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(54) Title: PURINERGIC MODULATION OF SMELL

(57) Abstract: Disclosed are compositions and methods for modulating odor sensitivity, as well as screening methods for detecting compounds that modulate odor sensitivity.

PURINERGIC MODULATION OF SMELL

I. CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims the benefit of U.S. provisional application Serial No. 60/428,140, filed November 21, 2002. This application is hereby incorporated by this reference in its entirety for all of its teachings.

II. ACKNOWLEDGEMENTS

This invention was made with government support under federal grants DC04953 and DC02994 awarded by the NIH and NIDCD. The Government has certain rights to this invention.

III. BACKGROUND

A longstanding dogma, based on lack of efferent synapses, is that odor sensitivity is not modulated at the level of the olfactory receptor neurons (ORNs). The sensation of smell occurs in part by the activation of smell receptors present on the ORNs. This activation begins through contact of the chemical signature responsible for the odor with a smell receptor on the ORN. There is a need to be able to modulate sensitivity to smell, to for example, decrease sensitivity to smell in noxious environments and increase sensitivity to smell for environments in which it is desirable to smell the odors. Disclosed are methods and compositions which modulate the sensitivity to odor responsiveness.

IV. SUMMARY

As embodied and broadly described herein, the disclosed compositions and methods, in one aspect, relate to the modulation of smell. It is to be understood that both the foregoing general description and the following detailed description are exemplary and explanatory only and are not restrictive.

V. BRIEF DESCRIPTION OF THE DRAWINGS

The accompanying drawings, which are incorporated in and constitute a part of this specification, illustrate several embodiments of the compositions and methods and together with the description, serve to explain the principles of the compositions and methods.

Figure 1 shows the identification of purinergic receptors in the olfactory epithelium (OE). Figure 1(A) shows RT-PCR analysis of $P2X_2$ and $P2Y_2$ mRNA in rat OE and bulb. The 643-bp product represents the $P2Y_2$ isoform; the 499-bp product represents the $P2X_{2\text{-}1}$ isoform, and the 292-bp product is the $P2X_{2\text{-}2}$ isoform. Control β-actin (867 bp) and neuron specific enolase (NSE; 753 bp) RT-PCR reactions are shown. +, Indicates reverse transcribed mRNA; −, indicates omission of reverse transcriptase. Figures 1(B, C) show neonatal mouse OE showing punctate $P2X_1$- and $P2X_4$-IR (green) in olfactory marker protein (OMP)-positive (red) axons and olfactory receptor neurons (ORNs; closed arrowheads) and in OMP-negative ORNs and basal cells (open arrowheads). SC, sustentacular cell layer; BC, basal cell layer; NL, nerve layer; C, cribriform plate; NB, nerve bundle. Figure 1(D) shows neonatal mouse P2Y2 receptor-IR (green) occurs in ORNs (closed arrowheads), in the sustentacular cell layer (open arrowheads), and in a Bowman's gland (BG, *). Figure 1(E) shows P2X1 receptor antibody preabsorption. (LP, lamina propria) All scale bars, 20 μM.

Figure 2 shows ATP evokes inward currents and increases intracellular Ca^{2+} in cultured mouse olfactory receptor neurons (ORNs). (A) Current responses to 10 μM ATP in two nystatin-patched ORNs held at -110 mV. Lower trace shows the ATP stimulus profile recorded separately with an open electrode. *Inset*, enlarged, compressed view of current from cell 1. (B) Confocal images from fluo-4 AM loaded ORNs taken before (left), and during (right) superfusion of 5 μM ATP. Scale bar, 50 μm. (C) Representative fluorescence (F) increases from cells a and b in (B) in response to ATP (1-10 μM). (D) Dose-response relation for maximum % ΔF/F increases, relative to 10 μM ATP (mean ± S.E.M.; n = 58 ORNs for each concentration; EC_{50} = 1.6 μM). (E) Representative traces from 2 ORNs that responded to ATP (10 μM; arrowhead) in normal Ca^{2+} and in 0 Ca^{2+} + EGTA (open bar).

Figure 3 shows that odor and purinergic receptor (P2R) agonists evoke increases in $[Ca^{2+}]_i$. See also Supplementary Information. Figures 3(A1-D4) show confocal images from a fluo-4 AM-loaded mouse olfactory epithelium (OE) slice during application of (A) odors (10 μM n-amyl acetate + 10 μM R-carvone), (B) 10 μM ATP, (C) 10 μM βγ-methylene ATP (βγ-MeATP), or (D) 10 μM UTP. Figures (A5-D5) show time course of odor- and P2R-agonist-evoked Ca^{2+} transients. Time points indicated by black triangles correspond to frame numbers in A1-D4. Representative odor-responsive olfactory receptor neurons (ORNs) are indicated by solid white triangles (a1-a4; 6/11 ORNs marked) and as solid lines in a5. One odor-responsive ORN (solid triangle in b1-d4) and one sustentacular cell (SC, open triangle in B1-D4) are shown in the time course (B5-D5).

Figure 4 shows a frequency of response to purinergics in ORNs and sustentacular cells. Shown are the percentages of ATP sensitive ORNs, Figure 4(A); identified by odor responsiveness; n = 14), and SCs, Figure 4(B); identified by location and lack of odor response; n = 122), that had increases in $[Ca^{2+}]_i$ evoked by non-selective purinergic receptor agonists (ATP, ATPγS), P2Y-selective agonists (UTP, ADP, MeSADP) and P2X-selective agonists (βγ-MeATP).

Figure 5 shows that ATP modulates odor responses. Figure 5(A) Suppression or Figure 5(B) enhancement of $[Ca^{2+}]_i$ due to co-application (Co-App.) compared to the summed response of ATP and odor. Shown are responses to odor (10 μM n-amyl acetate + 10 μM R-carvone), 10 μM ATP, control Ringers solution, or co-application of odor + ATP from individual mouse ORNs in olfactory epithelium slices. Figure 5(C) Bar graph showing suppression and enhancement from the 2 individual ORNs shown in A and B. The sum of the responses to individual application of ATP and odor were normalized to 1.0 (stacked bars) and the response to co-application of ATP and odor were normalized to the summed response (black bars).

Figure 6 shows the activation of specific purinergic receptor subtypes modulates odor responses. Figures 6 (A, C, E) Representative calcium transients in response to odor (10 μM n-amyl acetate + 10 μM R-carvone), 10 μM purinergic receptor (P2) agonists, or co-application of odor + P2 agonists from individual mouse ORNs in Fluo-4 AM loaded olfactory epithelium slices. Black triangles correspond to the time of loop injection of the odors or P2 agonists. Black circles correspond to the predicted peak amplitude of co-application (obtained by adding the estimated odor and P2 agonist values; refer to data analysis section for details). (B, D, F) Responses to individual application of P2 agonists and odor were normalized to the sum of

each response and averaged (stacked bars). The responses to co-application of P2 agonists and odor were normalized to the summed individual responses and averaged (black bars). The recoveries, obtained after co-application, were also normalized to the initial summed response. Bar graphs depict normalized peak Ca^{2+} transient amplitudes (mean + s.e.m.). **(A-B)** Co-application of 10 μM βγ-methylene ATP (βγ-MeATP) and odors enhanced the calcium transient amplitude in 2/16 ORNs from 2 slices. **(C-D)** Co-application of 10 μM βγ-MeATP suppressed the calcium transient amplitude in 12/16 ORNs from 6 slices. **(E-F)** Co-application of 10 μM ADPβS and odors reduced the calcium transient amplitude. N = 15 ORNs from 5 slices.

Figure 7 shows examples of the growing family of ATP-gated ion channels. The predicted primary amino acid sequences of cloned $P2X_1$-$P2X_6$ receptor subtypes show that these proteins share approximately 40% sequence identity (*gray shading*) overall. Ten invariant cysteine residues (*) located within the presumptive extracellular loop may be essential for stabilizing a ligand-binding pocket through the formation of specific disulfide bonds. Putative transmembrane -helices are delimited with black bars labeled M1 and M2. A potential pore loop region akin to that found in potassium channels corresponds to the portion of M2 denoted as (H5).

Figure 8 shows a diagram depicting a proposed transmembrane topology for P2X2 protein showing both N- and C-terminals in the cytoplasm. Two putative membrane spanning segments (M1 and M2) traverse the lipid bilayer of the plasma membrane and are connected by a hydrophilic segment of 270 amino acids. This putative extracellular domain is shown containing two disulfide-bonded loops (S-S) and three N-linked glycosyl chains (triangles). The P2X2 cDNA was sequenced on both strands using Sequanase. (From Brake et al., 1994).

Figure 9 shows a predicted secondary structure of the human P2Y1-receptor. Bold circles and letters highlight amino acids that most likely contribute to the nucleotide binding site within the transmembrane regions. A change of these residues by site-directed mutagenesis caused both an increase in half-maximal concentrations of agonists such as 2-methylthio-ADP activating phospholipase C (Jiang et al. 1997) and a reduction of the antagonistic potency of the nucleotide antagonist MRS 2179 (Moro et al. 1998). The dashed lines show predicted disulphide bridges (Hoffmann et al. 1999). Glu at the position 209 and Arg at the position 287 may form additional (probably low affinity) binding sites ("meta-binding sites"; see Moro et al. 1999). Potential sites for N-linked glycosylation are not indicated (TM transmembrane region, EL extracellular loop).

Figure 10 shows the alignment of the amino acid composition of the predicted transmembrane regions (*TMs*) 3, 5, 6 and 7 of the human $P2Y_1$-, $P2Y_2$-, $P2Y_4$-, $P2Y_6$- and $P2Y_{11}$-receptors (for each subtype, the principal physiological agonist is shown *in parentheses*; please note that the human $P2Y_2$-receptor is activated by both UTP and ATP). *Bold letters* show a pattern of similarity in amino acid composition, which may be responsible for the pharmacological properties of the subtype. The respective residues are conserved within species. *Underlined letters* indicate a reduction or loss in functional responses of the (human) $P2Y_1$- or (murine) $P2Y_2$-receptor after replacement of that residue by site-directed mutagenesis. *Italic letters* indicate that a replacement had failed to change the responses (see Erb et al. *1995*; Jiang et al. *1997*).

Figure 11 shows the chemical structure of some key agonists and antagonists at P2 receptors. (Adapted from Windscheif, 1996).

Figure 12 shows the results of the addition of antagonists and odor stimulants on nerve cells. Representative normalized calcium transients in response to odor in the absence (A) or presence (B) of P2 receptor antagonists (100 uM suramin 25 uM PPADS) from individual mouse ORNs in fluo-4-AM-loaded OE slices. Filled triangles correspond to the time of loop injection of the odors. Slices were pretreated for 3 min with Ringer's solution or P2 receptor antagonists (open columns). C, Average peak calcium transient amplitudes are shown (means + SEM), as are the predicted peak amplitudes (filled circles) for the second application (n=30 ORNs from seven slices for control and n=22 ORNs from 12 slices for P2 receptor antagonists). The asterisk indicates a significant increase in [Ca2+]i in the observed compared with predicted ($p<0.024$, paired Student's t test). D, Representative traces depicting basal fluorescence levels when bath is switched at 10 sec (open column) from Ringer's solution to either P2 receptor antagonists (solid lines) or Ringer's solution (dotted lines). The fluorometric signals shown are expressed as relative fluorescence change,_□F/F (F-F0)/F, where F0 is calculated from the linear rate of decay during the first 15 sec of the recording (F0 = mX + b). Thus, values of 0 represent no change in fluorescence and calcium levels, negative values represent decreases in calcium, and positive values represent increases in basal calcium levels.

Figure 13 shows ATP suppresses cyclic nucleotide-induced electrical responses in olfactory epithelium. (A) shows representative EOG responses from OE slices attributable to Ringer's solution, odor, and a cyclic nucleotide mixture (100 μM IBMX, 50 μM CPT-cAMP, and 50 μM 8-Br-cGMP). Filled triangles correspond to the time of loop injection of the test solutions. (B) shows representative on-cell current-clamp recording from an ORN in an OE slice. Various test solutions were superfused onto the slice for 30 seconds, indicated by the shaded region. The cell was allowed to recover for 7 minutes after each test application. Note that the coapplication of ATP (10 μM) and the mixture suppressed the evoked membrane potential changes. (C) shows the electrical activity from each ORN was integrated from baseline, normalized to the initial cyclic nucleotide mixture response, and averaged (means +SEM). *$p<0.05$, Newman-Keuls post hoc test. N=3 from three slices, also indicated in each column.

VI. DETAILED DESCRIPTION

The present compositions and methods can be understood more readily by reference to the following detailed description and the Examples included therein and to the Figures and their previous and following description.

Before the present compounds, compositions, articles, devices, and/or methods are disclosed and described, it is to be understood that the compositions and methods are not limited to specific synthetic methods, specific recombinant biotechnology methods unless otherwise specified, or to particular reagents unless otherwise specified, as such can, of course, vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only and is not intended to be limiting.

A. Definitions

As used in the specification and the appended claims, the singular forms "a," "an" and "the" include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to "a pharmaceutical carrier" includes mixtures of two or more such carriers, and the like.

Ranges can be expressed herein as from "about" one particular value, and/or to "about" another particular value. When such a range is expressed, another embodiment includes from the one particular value and/or to the other particular value. Similarly, when values are expressed as approximations, by use of the antecedent "about," it will be understood that the particular value forms another embodiment. It will be further understood that the endpoints of each of the ranges are significant both in relation to the other endpoint, and independently of the other endpoint. It is also understood that there are a number of values disclosed herein, and that each value is also herein disclosed as "about" that particular value in addition to the value itself. For example, if the value "10" is disclosed, then "about 10" is also disclosed. It is also understood that when a value is disclosed that "less than or equal to" the value, "greater than or equal to the value" and possible ranges between values are also disclosed, as appropriately understood by the skilled artisan. For example, if the value "10" is disclosed then "less than or equal to 10"as well as "greater than or equal to 10" is also disclosed.

"Optional" or "optionally" means that the subsequently described event or circumstance may or may not occur, and that the description includes instances where said event or circumstance occurs and instances where it does not.

"Primers" are a subset of probes which are capable of supporting some type of enzymatic manipulation and which can hybridize with a target nucleic acid such that the enzymatic manipulation can occur. A primer can be made from any combination of nucleotides or nucleotide derivatives or analogs available in the art, which do not interfere with the enzymatic manipulation.

"Probes" are molecules capable of interacting with a target nucleic acid, typically in a sequence specific manner, for example through hybridization. The hybridization of nucleic acids is well understood in the art and discussed herein. Typically a probe can be made from any combination of nucleotides or nucleotide derivatives or analogs available in the art.

"Coapplication" is defined as the application of one or more substances simultaneously, such as in the same formulation or consecutively, within a time frame such that each substance is active during a point when the other substance or substances are active.

The terms "higher," "increases," "elevates," or "elevation" refer to increases above basal levels, e.g., as compared to a control. The terms "low," "lower," "reduces," or "reduction" refer to decreases below basal levels, e.g., as compared to a control. For example, basal levels are normal *in vivo* levels prior to, or in the absence of, or addition of an agent such as an agonist or antagonist.

The term "test compound" is defined as any compound to be tested for its ability to interact with a purinergic receptor, e.g., an epithelial Ca^{2+} entry channel agonist or antagonist. Also, "test components" include,

for example, drugs, molecules, and compounds that come from combinatorial libraries where thousands of such ligands are screened by drug class.

The terms "control levels" or "control cells" are defined as the standard by which a change is measured, for example, the controls are not subjected to the experiment, but are instead subjected to a defined set of parameters, or the controls are based on pre- or post-treatment levels.

Throughout this application, various publications are referenced. The disclosures of these publications in their entireties are hereby incorporated by reference. The references disclosed are also individually and specifically incorporated by reference herein for the material contained in them that is discussed in the sentence in which the reference is relied upon.

Disclosed are the components to be used to prepare the disclosed compositions as well as the compositions themselves to be used within the methods disclosed herein. These and other materials are disclosed herein, and it is understood that when combinations, subsets, interactions, groups, etc. of these materials are disclosed that while specific reference of each various individual and collective combinations and permutation of these compounds may not be explicitly disclosed, each is specifically contemplated and described herein. For example, if a particular ATP analog is disclosed and discussed and a number of modifications that can be made to a number of molecules including the ATP analog are discussed, specifically contemplated is each and every combination and permutation of the ATP analog and the modifications that are possible unless specifically indicated to the contrary. Thus, if a class of molecules A, B, and C are disclosed as well as a class of molecules D, E, and F and an example of a combination molecule, A-D is disclosed, then even if each is not individually recited each is individually and collectively contemplated meaning combinations, A-E, A-F, B-D, B-E, B-F, C-D, C-E; and C-F are considered disclosed. Likewise, any subset or combination of these is also disclosed. Thus, for example, the sub-group of A-E, B-F, and C-E would be considered disclosed. This concept applies to all aspects of this application including, but not limited to, steps in methods of making and using the disclosed compositions. Thus, if there are a variety of additional steps that can be performed it is understood that each of these additional steps can be performed with any specific embodiment or combination of embodiments of the disclosed methods.

It is understood that the compositions disclosed herein have certain functions, such as enhancing or reducing odor sensitivity. Disclosed herein are certain structural requirements for performing the disclosed functions, and it is understood that there are a variety of structures which can perform the same function which are related to the disclosed structures, and that these structures will ultimately achieve the same result, for example stimulation or inhibition of smell.

B. Compositions and methods

Purinergic nucleotides are important neuromodulators of auditory and visual systems. Disclosed herein is the existence and activity of purinergic receptors in mammalian olfactory epithelium, such as mouse or human, determined through immunohistochemistry, electrophysiology and calcium imaging. P2X and P2Y receptors, such as P2Y2, P2X1 and P2X4 immunoreactivity (-IR) was present on the dendrites, soma and axons of olfactory marker protein$^+$ (OMP) ORNs, and in the olfactory nerve, glomular and mitral cell layers of

the olfactory bulb. In addition, P2Y2-IR was observed in the sustentacular cell layer of the epithelium. Application of ATP (10 μM) onto perforated patched mouse ORNs evoked inward currents with two distinct latent periods, indicating involvement of both rapidly activating ligand-gated P2X receptors and G-protein coupled P2Y receptors, which should have a slow onset of activation. Application of ATP (10 μM) evoked a rapid transient increase in intracellular calcium ($[Ca^{2+}]_i$). In the absence of external Ca^{2+}, ATP-evoked larger calcium transients than responses in the presence of Ca^{2+} indicating that at least part of the signal results from release from intracellular Ca^{2+} stores implicating P2Y receptor contribution to ATP-mediated Ca^{2+} transients. An olfactory epithelial (OE) slice preparation and confocal imaging was used to measure changes in $[Ca^{2+}]_i$ in fluo-4 acetoxymethyl ester (AM) loaded OE slices in response to odor and purinergic nucleotide application. Use of selective purinergic receptor agonists demonstrated that P2X and P2Y receptor agonists evoke increases in $[Ca^{2+}]_i$ in ORNs with equal frequency and that P2Y but not P2X receptor agonists evoke calcium transients in sustentacular cells. $[Ca^{2+}]_i$ levels were measured in response to odor, ATP, or odor + ATP. In most cells, ATP reduced the summed odor-induced changes in Ca^{2+} however, some cells exhibited an increase in evoked $[Ca^{2+}]_i$ increase, indicating an increased effect. Collectively, the data indicates that P2X and P2Y receptor subtypes are expressed in the olfactory epithelium and that P2X and P2Y agonists and antagonists modulation of odor responses, such as the agonist ATP, can be dependent on the subtype(s) of purinergic receptors expressed.

Disclosed herein is direct evidence that ATP and ATP analogs modulate odor responses in olfactory receptor neurons. ATP released in the olfactory epithelium following noxious stimuli provides a physiological source for a neuromodulatory substance independent of efferent innervation. Peripheral ATP-mediated odor suppression is a mechanism for reduced olfactory sensitivity during exposure to olfactotoxins. Methods for modulating the sensitivity to smell of a subject are disclosed.

1. P2X and P2Y purinergic receptors

P2X receptors form Ca^{2+}-permeable nonselective cation channels that allow Ca^{2+} influx from the extracellular fluid. Most of the 8 functional P2Y receptors identified to date act via G-protein coupling to activate phospholipase C, leading to production of inositol triphosphates and mobilization of Ca^{2+} from internal stores (Dubyak and el-Moatassim, 1993); however, a few P2Y receptors couple to adenylate cyclase (Ralevic and Burnstock, 1998). All of the components of both transduction pathways have been identified in ORNs (Schild and Restrepo, 1998).

Although purines are odorants for aquatic vertebrates (Kang and Caprio, 1995) and invertebrates (Carr, W. E., et al., Environ. Health Perspect. 71, 31-46 (1987)), disclosed herein, extracellular purinergic nucleotides and their receptors in mammalian, such as human, olfactory epithelium exist. Disclosed herein, RT-PCR and immunohistochemistry and physiological studies, show that sustentacular support cells express P2Y receptors and that ORNs express both P2X and P2Y receptors. Regionally localized purinergic receptors are consistent with extracellular ATP having multiple roles in the peripheral olfactory system. Furthermore, it is shown herein that ATP differentially modulates the odor responsiveness of ORNs. This indicates that the complement of P2X and/or P2Y receptor subtypes expressed in the ORN can determine whether the odor response is enhanced or inhibited in the presence of ATP.

There are two main families of purine receptors, adenosine or P1 receptors, and P2 receptors, recognizing primarily ATP, ADP, UTP, and UDP (Table 1). Adenosine/P1 receptors couple to G proteins and have been further subdivided, based on molecular, biochemical, and pharmacological evidence into four subtypes, A_1, A_{2A}, A_{2B}, and A_3. In contrast, P2 receptors divide into two families of ligand-gated ion channels and G protein-coupled receptors termed P2X and P2Y receptors, respectively. For example, Table 1 sets forth seven mammalian P2X receptors ($P2X_{1-7}$) and five mammalian P2Y receptors ($P2Y_1$, $P2Y_2$, $P2Y_4$, $P2Y_6$, $P2Y_{11}$) which have been cloned and characterized.

TABLE 1: **Families of receptors for purines and pyrimidines**

(Modified from Ralevic V, Burnstock G. Pharmacol Rev 1998 Sep;50(3):413-92.)

	Adenosine/P1 receptors	**P2 receptors**	
Natural ligands	Adenosine	ATP, ADP, UTP, UDP, Adenine dinucleotides	
Subgroup	—	**P2X**	**P2Y**
Type	G protein-coupled	Ion channel Nonselective pore	G protein-coupled
Subtypes	A_1, A_{2A}, A_{2B}, A_3	$P2X_{1-7}$, $P2X_n$	$P2Y_1$, $P2Y_2$, $P2Y_4$, $P2Y_6$, $P2Y_{11}$, $P2Y_{ADP}$ (or P_{2T}) Uridine nucleotide-specific

P2X receptors are ATP-gated ion channels which mediate rapid (within 10 ms) and selective permeability to cations (Na^+, K^+ and Ca^{2+}) (Bean, 1992; Dubyak and el-Moatassim, 1993; North, 1996). They are typically found on excitable cells (smooth muscle cells, neurons, and glial cells) and mediate fast excitatory neurotransmission to ATP in both the central and peripheral nervous systems. This contrasts with the slower response (less than 100 ms) to ATP acting at metabotropic P2Y receptors, which involves coupling to G proteins and second-messenger systems. Seven functional P2X receptor proteins ($P2X_1$ to $P2X_7$) have been cloned and form homomeric ion channels with distinct pharmacological profiles when expressed in *Xenopus* oocytes (Table 2). The $P2X_7$ receptor is considered separately below, because it is functionally unique among P2X receptors in being able to act as a non-selective pore.

Functional cDNAs encoding the first two members of this family, $P2X_1$ and $P2X_2$, were isolated from vas deferens smooth muscle and PC12 pheochromocytoma cells, respectively, using an expression cloning strategy in *Xenopus* oocytes (Brake et al 1994, Valera et al 1994). In each case, expression of a single cDNA clone in oocytes or transfected mammalian cells is sufficient to direct the synthesis of functional, presumably homomeric ATP-gated ion channel complexes on the surface of these cells. $P2X_1$ and $P2X_2$ receptors are clearly related at the level of primary amino acid sequence and predicted secondary structure. Four additional members of this channel family have now been cloned using PCR-based screening strategies (Bo et al 1995, Chen et al 1995, Lewis et al 1995, Buell et al 1996, Collo et al 1996, Seguela et al 1996). All six subtypes share approximately 40% sequence identity distributed fairly evenly over their length, which ranges from 379 to 472 residues (Figure 7).

TABLE 2 **Cloned P2X receptors and typical activity profiles**

Receptor	Acession number	cDNA library source	Agonist activity	References
$P2X_1$	X83688	Human urinary bladder	ATP> α,β-meATP	Evans *et al.*, 1994; Valera *et al.*, 1995; Longhurst *et al.*, 1996
(399 amino acids (aa))	X80477	Rat vas deferens	2MeSATP > ATP > α,β-meATP	Valera *et al.*, 1994
	X84896	Mouse urinary bladder		Valera *et al.*, 1996
$P2X_2$ (472 aa)	U14414	Rat PC12 cells	2MeSATP > ATP; α,β-meATP inactive	Brake *et al.*, 1994
$P2X_{2(b)}$[a] (401 aa)	Y09910	Rat cerebellum	2MeSATP = ATP = α,β - meATP	Brändle *et al.*, 1997; Simon *et al.*, 1997
$P2X_3$ (397 aa)	Y07684	Human heart, spinal cord	2MeSATP > ATP > α,β - meATP	Garcia-Guzman *et al.*, 1997b
	X90651	Rat dorsal root ganglion cells	2MeSATP > ATP > α,β-meATP > UTP	Chen *et al.*, 1995a
	X91167	Rat dorsal root ganglion cells	ATP > 2MeSATP > α,β-meATP	Lewis *et al.*, 1995
$P2X_4$ (388 aa)	Y07684	Human brain	ATP » 2MeSATP ≥ CTP > α,β-meATP	Garcia-Guzman *et al.*, 1997a
	X93565	Rat brain	ATP » 2MeSATP ≥ CTP > α,β-meATP	Soto *et al.*, 1996a
	U32497	Rat brain	ATP > 2MeSATP » α,β-meATP	Séguéla *et al.*, 1996
	X91200	Rat hippocampus	ATP > 2MeSATP » α,β-meATP	Bo *et al.*, 1995
	X87763	Rat superior cervical ganglion	ATP; α,β-meATP inactive	Buell *et al.*, 1996b
	U47031	Rat pancreatic islet	ATP, ADP, 2MeSATP » α,β-meATP	Wang *et al.*, 1996
$P2X_5$ (417 aa)	X92069	Rat ganglia	ATP > 2MeSATP > ADP α,β-meATP inactive	Collo *et al.*, 1996
(455 aa)	X97328	Rat heart	ATP > 2MeSATP > ADP	Garcia-Guzman *et al.*, 1996
$P2X_6$	X92070	Rat superior cervical ganglion	ATP > 2MeSATP > ADP; α,β-meATP inactive	Collo *et al.*, 1996
(379 aa)	X97376	Rat brain		Soto *et al.*, 1996b
$P2X_7$ (595 aa)		Mouse macrophage	BzATP > ATP > UTP ATP > UTP > BzATP	Nuttle *et al.*, 1993
	X95882	Rat macrophage and brain	BzATP > ATP > 2MeSATP > ADP; UTP inactive	Surprenant *et al.*, 1996
		Human monocytes	BzATP > ATP	Rassendren *et al.*, 1997

[a] Splice variant, also termed $P2X_{2-2}$.

All references are herein incorporated by reference at least for material related to a P2X or P2Y receptor.

Modified from Ralevic V, Burnstock G. Pharmacol Rev 1998 Sep;50(3):413-92.

Based on the amino acid sequences of cloned P2X receptor subunits, structural features of P2X receptors have been predicted. The P2X proteins that have been cloned are receptor subunits, not actual receptors since a single 2 transmembrane subunit alone cannot form an ion channel. The proteins have 379 to 472 amino acids and are believed to insert into the cell membrane to form a pore comprising two hydrophobic transmembrane domains (M1 and M2), with much of the protein occurring extracellularly as an intervening hydrophilic loop (figure 8). It is presumed that both amino- and carboxyl-termini are located on the intracellular side of the membrane. Based on genetic studies in *C. elegans*, the M2 domain of these channels forms an amphipathic α -helix whose hydrophilic face lines the pore (Hong & Driscoll 1994). Interestingly, helical wheel plots of M2 domains from each of the cloned P2X subunits show that they have similar potential to form amphipathic α-helices, despite the limited sequence homology in this region. In addition, some P2X subunits contain a region, that resembles the H5 pore loop domain of potassium channels, and it is possible that this segment (just amino-terminal to M2), also contributes to the pore of ATP-gated channels. However, there is considerable variability in the H5 domain consensus sequence, and its location relative to M2, among the six cloned P2X receptor subtypes. The overall structure of the receptor most closely resembles that of amiloride-sensitive epithelial Na^+ channels. The putative extracellular loop of cloned receptors $P2X_1$ to $P2X_7$ has 10 conserved cysteine residues, 14 conserved glycine residues and 2 to 6 potential N-linked glycosylation sites. It is believed that disulfide bridges may form the structural constraints needed to couple the ATP-binding site to the ion pore. Most of the conserved regions are in the extracellular loop, with the transmembrane domains being less well-conserved. As for other ligand-gated receptors, P2X receptors are believed to form a heterologous complex in biological tissues. Although their subunit stoichiometry is unknown, SDS polyacrylamide gel electrophoresis estimates of the relative molecular mass of the recombinant $P2X_1$ and $P2X_3$ receptors determined under non-denaturing conditions (Nicke *et al.*, 1998) suggest a combination of three subunits (or multiples of three subunits).

Both cloned $P2X_7$ and endogenous $P2X_7$-like receptors are unique in that, under physiological conditions they are selectively permeable to small cations only, but in the presence of low divalent cation levels and ATP, the $P2X_7$ channel can convert to a pore, permeable to small molecules as well as ions. The $P2X_7$ receptor and its endogenous counterpart is structurally similar to other P2X receptors, except for the fact that it has a significantly longer intracellular C-terminal (240 amino acids) than other P2X receptors, of which at least the last 177 amino acids are crucial for the induction of the non-selective pore (Surprenant *et al.*, 1996). Brief activation of the recombinant $P2X_7$ receptor and its endogenous counterpart causes rapid membrane depolarization and cation influx and is a reversible process. However, sustained activation causes an increase in permeability by allowing bidirectional transport of a variety of ions including Na^+, K^+, and Ca^{2+} and small molecules with a molecular weight of less than or equal to 900 daltons, except in lymphocytes where the limit is 200-300 daltons. This effect is associated with cytotoxicity. Although cation function of the receptor is retained in a truncated $P2X_7$ receptor lacking the last 177 residues, the increased permeability is lost suggesting involvement of the cytoplasmic C terminus. The disclosed results indicate that the $P2X_7$ receptor is not typically present in mammalian olfactory epithelium.

P2Y receptors are purine and pyrimidine nucleotide receptors that are coupled to G proteins. Most P2Y receptors act via G protein coupling to activate PLC leading to the formation of IP_3 and mobilization of intracellular Ca^{2+}. Coupling to adenylate cyclase by some P2Y receptors has also been described. The response time of P2Y receptors is longer than that of the rapid responses mediated by P2X receptors because it involves second-messenger systems and/or ionic conductances mediated by G protein coupling. Five mammalian P2Y receptors ($P2Y_1$, $P2Y_2$, $P2Y_4$, $P2Y_6$, $P2Y_{11}$) have been cloned, and functionally characterized and show distinct pharmacological profiles (Table 3).

TABLE 3: **Cloned P2Y receptors**

Receptor	Acession number	cDNA library source	Agonist activity	References
$P2Y_1$		Human brain	2MeSATP > ATP » UTP	Schachter *et al.*, 1996
(362 amino acids (aa))	S81950	Human prostate and ovary	2MeSATP > ATP = ADP	Janssens *et al.*, 1996
	Z49205	Human placenta		Léon *et al.*, 1995, 1997
	U42030	Human HEL cells		Ayyanathan *et al.*, 1996
	X87628	Bovine endothelium	2MeSATP = ADP > ATP » UTP	Henderson *et al.*, 1995
	U22830	Rat insulinoma cells	2MeSATP > 2Cl-ATP > ATP (α,β-meATP inactive)	Tokuyama *et al.*, 1995
		Rat ileal myocytes	2MeSATP = 2ClATP > ADP > ATP (UTP inactive)	Pacaud *et al.*, 1996
	U22829	Mouse insulinoma cells		Tokuyama *et al.*, 1995
	U09842	Turkey brain	2MeSATP > ADP > ATP; (UTP inactive)	Filtz *et al.*, 1994
	X73268	Chick brain	2MeSATP > ATP > ADP; (UTP inactive)	Webb *et al.*, 1993b
$P2Y_2$	U07225	Human CF/T43 epithelial cells	ATP = UTP » 2MeSATP	Parr *et al.*, 1995
(373 aa)		Human bone		Bowler *et al.*, 1995
		Rat microvascular coronary EC		Gödecke *et al.*, 1996
	U09402	Rat alveolar type II cells	ATP = UTP	Rice *et al.*, 1995
	L46865	Rat pituitary	ATP = UTP > ADP = UDP > GTP	Chen *et al.*, 1996b
	U56839	Wistar Kyoto rat[a]		Seye *et al.*, 1996
	NM_008773	Mouse NG108-15 neuroblastoma cells	ATP = UTP > ATPγS » 2MeSATP	Lustig *et al.*, 1993
p2y3[b] (328 aa)	X98283	Chick brain	UDP > UTP > ADP > 2MeSATP > ATP	Webb *et al.*, 1995, 1996a
$P2Y_4$	X91852	Human placenta	UTP > ATP = ADP[c]	Communi *et al.*, 1996b
(352 aa)		Human placenta		Stam *et al.*, 1996
	U40223	Human chromosome X	UTP > UDP (ATP inactive)	Nguyen *et al.*, 1996
	Y14705	Rat heart	ATP = UTP = ADP = ITP = ATPγS = 2MeSATP = Ap_4A > UDP	Bogdanov *et al.*, 1998
$P2Y_6$	X97058	Human placenta and spleen	UDP > UTP > ADP > 2MeSATP » ATP	Communi *et al.*, 1996b
(379 aa)	NM_057124	Rat aortic smooth muscle	UTP > ADP = 2MeSATP > ATP	Chang *et al.*, 1995
	U52464	Activated T-cells		Southey *et al.*, 1996
$P2Y_{11}$ (371 aa)	371	Human placenta	ATP > 2MeSATP >>> ADP; (UTP, UDP inactive)	Communi *et al.*, 1997

[a] Tissue not specified.

[b] p2y3 may be the chick homologue of the mammalian $P2Y_6$ receptor.

[c] The reported activity of UDP at the $P2Y_4$ receptor has been shown to be caused by UTP present as a contaminant.

Each of the references herein is incorporated by reference at least for material related to P2Y receptors

Modified from Ralevic V, Burnstock G. Pharmacol Rev 1998 Sep;50(3):413-92.

P2Y receptors are 308 to 377 amino acid proteins with a mass of 41 to 53 kDa after glycosylation. The tertiary structure of P2Y receptors is similar to that of other seven transmembrane domain G protein-coupled receptors (Figure 9). A model of the P2Y receptor, based on the primary sequence of the $P2Y_1$ receptor and using the structural homolog rhodopsin as a G protein-coupled receptor template, has identified positively charged amino acid residues in transmembrane regions 3, 6, and 7 that may be involved in ligand binding by electrostatic interactions with the phosphates of ATP (Van Rhee *et al.*, 1995) (Figure 10). Several of these amino acids are conserved in other G protein-coupled receptors. Site-directed mutagenesis of the $P2Y_2$ receptor to convert positively charged amino acids in transmembrane regions 6 and 7 to neutral amino acids causes a 100- to 850-fold decrease in the potency of ATP and UTP, which suggests a role for these amino acids in binding purines and pyrimidines (Erb *et al.*, 1995). In contrast, in the human $P2Y_1$ receptor, the most important residues for ATP binding are in transmembrane regions 3 and 7 on the exofacial side of the receptor (Jiang *et al.*, 1997).

2. ATP and ATP analog activity on purinergic receptors

Extracellular ATP plays an important role in cellular signaling and acts as a cotransmitter or neuromodulator in sensory systems (Thorne and Housley, 1996). In the olfactory system, ATP can be released from synaptic vesicles in trigeminal afferents that innervate the olfactory epithelium and detect noxious chemicals (Finger et al., 1990;Getchell and Getchell, 1992), or via plasma membrane nucleotide transport proteins (Roman et al., 1997). Furthermore, ischemic, stressed, and injured cells release ATP in large amounts. A recent toxicology study (Kilgour et al., 2000) showed that when the olfactory epithelium was damaged by noxious fumes $[ATP]_i$ significantly decreased, whereas stimulation that did not damage the olfactory epithelium did not affect $[ATP]_i$. In addition to toxic chemicals, prolonged exposure to concentrated odors, such as peppermint, will damage olfactory receptor neurons (ORNs) and induce expression of stress indicators (heat shock proteins) in sustentacular support cells (Carr et al., 2001). Therefore, both trigeminal and odorous stimulation provide sources for extracellular ATP in olfactory epithelium.

Once released, ATP can have autocrine or paracrine effects. Very low concentrations of ATP activate the two subtypes (P2X and P2Y) of purinergic receptors (0.1-10 μM) (Ralevic and Burnstock, 1998;Schwiebert and Kishore, 2001). Through either of these receptor subtypes, ATP is able to stimulate an increase in $[Ca^{2+}]_i$ (Illes et al., 2000;Koshimizu et al., 2000;Ralevic and Burnstock, 1998).

P2 receptors have broad natural ligand specificity, recognizing ATP, ADP, UTP, UDP, and the diadenosine polyphosphates (Table 1). The chemical structures of some principal P2 receptor agonists and antagonists are illustrated in Figure 11. For example, P2X selective agonists are the stable ATP analogs α,β-meATP and β,γ-meATP, which if effective, strongly imply actions at P2X receptors (typically at $P2X_1$ and $P2X_3$ subtypes) and are generally inactive at P2Y receptors. Also useful are ADP, adenosine 5'-O-(2-thiodiphosphate)(ADPβS,) and UTP, as these are agonists at some P2Y receptors, but are weak or inactive at P2X receptors.

TABLE 4 **Exemplary P2 receptor signal transduction mechanisms, agonists, and antagonists**

Family	P2X	P2Y
Receptor type	Ion channel: Nonselective pore[a]	G protein-coupled: $G_{q/11}$, G_i[b]
Signaling pathway	Not applicable	PLC, AC[c] K^+ channels[d], PLC_{PC},[e] PLA_2,[f] PLD[f], PKC, MAPK[g]
Effectors	$Ca^{2+} » Na^+ > K^+$	$\uparrow IP_3$, $\uparrow Ca^{2+}$, $\uparrow$DAG $\downarrow$cAMP[c], Ca^{2+}, Cl, K^+ currents[h]
Nonselective Agonists	ATP[i], ATPγS, 2MeSATP, Ap_4A[j]	ATP[i], ATPγS, 2MeSATP, Ap_4A[j]
P2X/P2Y-selective Agonists	α,β-meATP[l], β,γ-meATP[l], BzATP[a]	ADP[c], UTP[m], UTPγS[j], UDP[n], 2Cl-ADP[c], 2MeSADP[c], ADPβS[c], ADPβF[c]
Nonselective Antagonists	Suramin, PPADS, Iso-PPADS, P5P, Reactive blue 2, Reactive Red, Trypan Blue, Evans Blue, DIDS	Suramin, PPADS, Iso-PPADS, P5P, Reactive blue 2, Reactive Red, Trypan Blue, Evans Blue, DIDS
P2X/P2Y-selective Antagonists	NF023, NF279, KN-62[a]	ARL 67085°, FPL 66096°, A3P5PS[k], MRS 2179[k], 2-hexylthio-ATP[p], 2-cyclohexylthio-ATP[p]

[a] $P2X_7$ and endogenous $P2X_7$-like receptor.
[b] $P2Y_1$ and endogenous $P2Y_1$-like receptors acting through PLC couple to $G_{q/11}$ proteins; $P2Y_1$ and endogenous $P2Y_1$-like receptors acting through adenylate cyclase couple to G_i proteins; $P2Y_2$ and endogenous $P2Y_2$-like receptors, $P2Y_4$ and $P2Y_{ADP}$ receptors couple to $G_{q/11}$ and G_i proteins; p2y3 and $P2Y_6$ receptors couple to $G_{q/11}$ proteins.
[c] $P2Y_1$ and endogenous $P2Y_1$-like receptors and $P2Y_{ADP}$ receptors.
[d] Some endogenous $P2Y_1$-like receptors activate K^+ channels via interactions with their G protein subunits.
[e] $P2Y_1$ and endogenous $P2Y_1$-like receptor signaling; possibly downstream of PKC.
[f] $P2Y_1$ and $P2Y_2$ receptors and their endogenous counterparts; signaling possibly downstream of PKC.
[g] $P2Y_1$ and $P2Y_2$ receptors and their endogenous counterparts; signaling downstream of PKC.
[h] Secondary to activation of PLC, although activation of K^+ currents by some endogenous $P2Y_1$-like receptors is via direct interactions with G protein subunits.
[i] $P2Y_1$ and $P2Y_2$ receptors and their endogenous counterparts; ATP is an antagonist at $P2Y_{ADP}$ receptors.
[j] $P2Y_2$ and endogenous $P2Y_2$-like receptors.
[k] $P2Y_1$ and endogenous $P2Y_1$-like receptors.
[l] $P2X_1$, $P2X_3$ and heteromeric $P2X_2P2X_3$ receptors.
[m] $P2Y_2$ and endogenous $P2Y_2$-like receptors and $P2Y_4$ receptors.
[n] $P2Y_6$ receptor. ° $P2Y_{ADP}$. [p] $P2Y_1$ and endogenous $P2Y_1$-like receptors coupled to AC.
Abbreviations: AC, adenylate cyclase; ADPβF, adenosine 5'-*O*-(2-fluoro)-diphosphate; ADPβS, adenosine 5'-*O*-(2-thio-diphosphate; cAMP, adenosine 3',5'-cyclic monophosphate; A3P5PS, adenosine 3'-phosphate 5'-phosphosulfate; ARL 67085,6-*N*,*N*-diethyl-D-β,γ-dibromomethylene ATP; ATPγS, adenosine 5'-*O*-(3-thiotriphosphate); BzATP, 3'-*O*-(4-benzoyl)benzoyl ATP; DAG, diacylglycerol; DIDS, 4,4'-diisothiocyanatostilbene-2,2'-disulfonate; FPL 66096, 2-propylthio-D-β,γ-difluoromethylene ATP; IP_3, inositol 1,4,5-trisphosphate; KN-62, 1-[*N*,*O*-bis(5-isoquinolinesulfonyl)-*N*-methyl-L-tyrosyl]-4-phenylpiperazine; Iso-PPADS, pyridoxal phosphate-6-azophenyl-2',5'-disulfonic acid; MAPK, mitogen-activated protein kinase; α,β-meATP, α,β -methylene ATP; β,γ-meATP, β,γ-methylene ATP; 2MeSADP, 2-methylthio ADP; 2MeSATP, 2-methylthio ATP; MRS 2179, N^6-methyl modification of 2'-deoxyadenosine 3',5'-*bis*phosphate; NF023, symmetrical 3'-urea of 8-(benzamido)naphthalene-1,3,5-trisulfonic acid; NF279, 8,8'-(carbonylbis(imino-4,1-phenylenecarbonylimino-4,1-phenylenecarbonylimino))bis(1,3,5-naphthalenetrisulfonic acid); P5P, pyridoxal-5-phosphate; PLC_{PC}, phosphatidylcholine-specific phospholipase C; PKC, protein kinase C; PLA_2, phospholipase A_2; PLC, phospholipase C; PLD, phospholipase D; PPADS, pyridoxal phosphate-6-azophenyl-2',4'-disulfonic acid; suramin, 8-(3-benzamido-4-methylbenzamido)-naphthalene-1,3,5-trisulfonic acid; UTPγS, uridine 5'-*O*-(3-thiotriphosphate).
Modified from Ralevic V, Burnstock G. Pharmacol Rev 1998 Sep;50(3):413-92.

3. Inhibiting olfactory response

As discussed herein if in an odor-ATP or analog assay, the calcium transient evoked by co-application is less than the sum of the calcium transients evoked by the individual components then there is an inhibiting

effect on the olfactory response. Of the cells that responded to odor, 62% (21/26 cells) exhibited a significant decrease in the summed $[Ca^{2+}]_i$ increase. The mean suppression of all cells was 57% ± 5% (paired t-test, p = 0.01, n = 26). Thus, ATP reduced the expected combined effect of the ATP and the odor, and thus will act as an odor suppressant. Typically, activation of P2Y receptors reduced sensitivity to odors. For example, the P2Y selective agonists UTP and ADP-βS suppressed the co-application evoked calcium transient indicating they can act as odor suppressants. As discussed in the Examples, similar experiments were performed with P2X and P2Y selective agonists giving similar results.

Disclosed are compositions and methods for inhibiting the odor response of an ORN. Inhibition of the response can be determined by performing the transient calcium flux assays as discussed herein. Typically these assays can be performed in the presence or absence of the odor. Thus, compositions which inhibit the ORN response can be compositions which in a calcium transient flux assay, the presence of the composition and the odor together, produces a transient calcium flux that is less than the sum of the odor induced flux alone and the composition induced flux alone. For example, if the amount of calcium flux in the presence of a composition and an odor is A, and the amount of the calcium flux in the presence of the composition alone is B and the amount of the calcium flux in the presence of the odor alone is C then if A< B+C, the composition can be said to inhibit the ORN response and the composition can inhibit a smell response. Disclosed are compositions wherein the combined effect of the composition and odor (A) is less than or equal to 95%, 90%, 85%, 80%, 75%, 70%, 65%, 60%, 55%, 50%, 45%, 40%, 35%, 30%, 25%, 20%, 15%, 10%, 5%, or 1% of the summed effect (B+C). It is understood that these numbers can be averages, with variances, and that these types of statistics can be employed, as discussed herein, to determine if the combined effect is less than the summed effect.

It is also understood that when the combined effect A, is less than the summed effect (B+C) that this can be expressed as a ratio of A/(B+C) and that ratios less than 1 indicate compositions that inhibit the ORN effect. For example, disclosed are compositions that have a ratio of less than or equal to 0.01, 0.03, 0.05, 0.07, 0.08, 0.09, 0.1, 0.2, 0.3, 0.4, 0.5, 0.52, 0.55, 0.6, 0.64, 0.65, 0.69, 0.7, 0.72, 0.75, 0.80, 0.83, 0.85, 0.87 0.90, 0.92, 0.95, 0.97, or 0.99. The ratio can be expressed in terms of a range of these individual ratios, such as 0.72 to 0.92, for example, or 0.52 to 0.64, or 0.69 to 0.83.

Thus, when an ORN is expressing the P2Y receptor and a P2Y selective agonist or a non-selective purinergic agonist is applied, odor response is suppressed. Likewise, when an ORN is expressing a P2X receptor and a P2X selective agonist is applied, the odor response is typically suppressed. Also, when both P2X and P2Y receptors are present on an ORN, and either a P2Y selective agonist, a P2X selective agonist, or a nonselective agonist is applied, the odor response is suppressed. Combinations of selective and non-selective agonists can be applied, and P2X and P2Y receptors can be suppressed depending on the combination of agonists in the mixture.

Disclosed are P2X selective agonists and P2Y selective agonists. Disclosed are P2X directed agonists and P2Y directed agonists. In certain embodiments, a P2X directed agonist is any agonist that has a greater effect on a P2X receptor than on a P2Y receptor. Likewise, in certain embodiments, a P2Y directed agonist is any agonist that has a greater effect on a P2Y receptor than on a P2X receptor. In other embodiments, P2X

agonists and P2Y agonists can be determined by comparing the activity to known selective agonists, such as those discussed herein. It is understood that the level of activity of each selective agonist discussed herein, is disclosed. Also disclosed are P2 agonists that interact with any P2 receptor. It is understood that many P2X and P2Y agonists can be both a selective agonist as well as a directed agonist. For example, UTP can be a selective and a directed P2Y agonist.

Just as P2X and P2Y agonists inhibit ORN response to odor stimulants, so too, antagonists of P2X and P2Y receptors can lead to an enhancement of the smell response. P2X antagonists, such as those disclosed in Table 4, for example, act at P2X receptors and P2Y antagonists, such as those disclosed in Table 4, for example, act at P2Y receptors, and thus can be stimulators of odor responsiveness. It is understood that the assays, measurements, and functional limitations, as discussed, herein for agonists are applicable for antagonists as well. Thus, for example, antagonists can be assayed in a calcium flux assay, but an antagonist would be considered a composition (B) that does not evoke a response in the calcium flux assay alone, i.e., B=0. However, when a composition (B) is co-applied with an odor (C), the combined odor and composition effect (A), would be greater than the effect of odor alone (C), or composition alone (B) and thus, A>(B+C) or since B=0, A> C.

Typically, antagonists have an opposite effect on a receptor than an agonist, and application of the disclosed methods and limitations can be thus applied to antagonists, as they were for agonists.

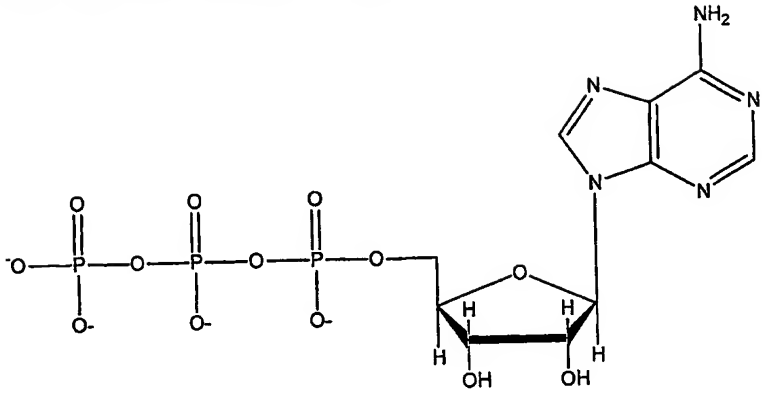
Disclosed are P2X selective antagonists and P2Y selective antagonists. Disclosed are P2X directed antagonists and P2Y directed antagonists. In certain embodiments, a P2X directed antagonist is any antagonist that has a greater effect on a P2X receptor than on a P2Y receptor. Likewise, in certain embodiments, a P2Y directed antagonist is any antagonist that has a greater effect on a P2Y receptor than on a P2X receptor. In other embodiments, P2X antagonist and P2Y antagonist can be determined by comparing the activity to known selective antagonists, such as those discussed herein. It is understood that the level of activity of each selective antagonist discussed herein, is disclosed. Also disclosed are P2 antagonists that interact with any P2 receptor. It is understood that many P2X and P2Y antagonists can be both a selective antagonist as well as a directed antagonists.

Disclosed herein are methods of modulating odor sensitivity in a subject, comprising administering a composition to the subject, wherein the composition is an antagonist of a P2X or P2Y purinergic receptor. The antagonist can increase the odor sensitivity of the subject, which can be desirable to those with olfactory impairments. Increasing odor sensitivity is also desirable in conjunction with a pleasant smell. The antagonist can reduce basal Ca^{2+} levels in olfactory receptor neurons which will make the neurons more excitable during subsequent odor stimulation thereby increasing the odor sensitivity of the subject. The antagonist can increase the ratio of observed coapplication-evoked calcium transient compared to the individual odor peak amplitudes in a cell activation assay, as discussed above.

C. Compositions

1. ATP and ATP analogs

The structure of ATP is shown in Formula 1.



Formula 1

There are many analogs of ATP that can be made. For example, analogs can be made at the base moiety, the sugar moiety, and the phosphate moiety, as discussed herein. The base moiety can be considered as adenin-9-yl (A). Many modifications can take place at this moiety. The sugar moiety of a nucleotide is typically a ribose or a deoxyribose. The phosphate moiety of a nucleotide is typically pentavalent phosphate. A non-limiting example of a nucleotide would be 3'-AMP (3'-adenosine monophosphate), ADP, and ATP.

ATP analogs can have modifications to the base moiety which would include natural and synthetic modifications of A, such as hypoxanthin-9-yl (I), and 2-aminoadenin-9-yl, 2-aminoadenine, xanthine, 6-methyl and other alkyl derivatives of adenine, 2-propyl and other alkyl derivatives of adenine, 8-halo, 8-amino, 8-thiol, 8-thioalkyl, 8-hydroxyl and other 8-substituted adenines, 7-methyladenine, 8-azaadenine, 7-deazaadenine and 3-deazaadenine, and O-6 substituted adenines, including 2-aminopropyladenine.

ATP analogs can also include modifications of the sugar moiety. Modifications to the sugar moiety would include natural modifications of the ribose and deoxy ribose as well as synthetic modifications. Sugar modifications include but are not limited to the following modifications at the 2' position: OH; F; O-, S-, or N-alkyl; O-, S-, or N-alkenyl; O-, S- or N-alkynyl; or O-alkyl-O-alkyl, wherein the alkyl, alkenyl and alkynyl can be substituted or unsubstituted C_1 to C_{10}, alkyl or C_2 to C_{10} alkenyl and alkynyl. 2' sugar modifications also include but are not limited to $-O[(CH_2)_n O]_m CH_3$, $-O(CH_2)_n OCH_3$, $-O(CH_2)_n NH_2$, $-O(CH_2)_n CH_3$, $-O(CH_2)_n -ONH_2$, and $-O(CH_2)_n ON[(CH_2)_n CH_3)]_2$, where n and m are from 1 to about 10.

ATP analogs can have other modifications at the 2' position and include but are not limited to: C_1 to C_{10} lower alkyl, substituted lower alkyl, alkaryl, aralkyl, O-alkaryl or O-aralkyl, SH, SCH_3, OCN, Cl, Br, CN, CF_3, OCF_3, $SOCH_3$, $SO_2 CH_3$, ONO_2, NO_2, N_3, NH_2, heterocycloalkyl, heterocycloalkaryl, aminoalkylamino, and polyalkylamino. Modified sugars would also include those that contain modifications at the bridging ring oxygen, such as CH_2 and S. Nucleotide sugar analogs can also have sugar mimetics such as cyclobutyl moieties in place of the pentofuranosyl sugar. There are numerous United States patents that teach the preparation of such modified sugar structures such as 4,981,957; 5,118,800; 5,319,080; 5,359,044; 5,393,878; 5,446,137; 5,466,786; 5,514,785; 5,519,134; 5,567,811; 5,576,427; 5,591,722; 5,597,909; 5,610,300;

5,627,053; 5,639,873; 5,646,265; 5,658,873; 5,670,633; and 5,700,920, each of which is herein incorporated by reference in its entirety.

ATP analogs can also be modified at the phosphate moiety. Modified phosphate moieties include but are not limited to those that can be modified so that the linkage between two nucleotides contains a phosphorothioate, chiral phosphorothioate, phosphorodithioate, phosphotriester, aminoalkylphosphotriester, methyl and other alkyl phosphonates including 3'-alkylene phosphonate and chiral phosphonates, phosphinates, phosphoramidates including 3'-amino phosphoramidate and aminoalkylphosphoramidates, thionophosphoramidates, thionoalkylphosphonates, thionoalkylphosphotriesters, and boranophosphates.

It is understood that nucleotide analogs need only contain a single modification, but can also contain multiple modifications within one of the moieties or between different moieties.

Nucleotide substitutes are molecules having similar functional properties to nucleotides, but which do not contain a phosphate moiety, such as peptide nucleic acid (PNA). Nucleotide substitutes are molecules that will recognize nucleic acids in a Watson-Crick or Hoogsteen manner, but which are linked together through a moiety other than a phosphate moiety. Nucleotide substitutes are able to conform to a double helix type structure when interacting with the appropriate target nucleic acid.

Nucleotide substitutes are nucleotides or nucleotide analogs that have had the phosphate moiety and/or sugar moieties replaced. Nucleotide substitutes do not contain a standard phosphorus atom. Substitutes for the phosphate can be for example, short chain alkyl or cycloalkyl internucleoside linkages, mixed heteroatom and alkyl or cycloalkyl internucleoside linkages, or one or more short chain heteroatomic or heterocyclic internucleoside linkages. These include those having morpholino linkages (formed in part from the sugar portion of a nucleoside); siloxane backbones; sulfide, sulfoxide and sulfone backbones; formacetyl and thioformacetyl backbones; methylene formacetyl and thioformacetyl backbones; alkene containing backbones; sulfamate backbones; methyleneimino and methylenehydrazino backbones; sulfonate and sulfonamide backbones; amide backbones; and others having mixed N, O, S and CH_2 component parts. Numerous United States patents disclose how to make and use these types of phosphate replacements and include but are not limited to 5,034,506; 5,166,315; 5,185,444; 5,214,134; 5,216,141; 5,235,033; 5,264,562; 5,264,564; 5,405,938; 5,434,257; 5,466,677; 5,470,967; 5,489,677; 5,541,307; 5,561,225; 5,596,086; 5,602,240; 5,610,289; 5,602,240; 5,608,046; 5,610,289; 5,618,704; 5,623,070; 5,663,312; 5,633,360; 5,677,437; and 5,677,439, each of which is herein incorporated by reference.

Disclosed are uses for non-selective, P2X selective and P2Y selective ATP analogs. Furthermore, there are ATP selective agonists and ATP selective antagonists. For example, non-selective purinergic receptor agonists are ATP, ATPγS, and AMP (Table 4). For example, P2Y-'selective' agonists are UTP, ADP, and MeS-ADP (Table 4). In addition, an example of a P2X-'selective' agonist is βγ-methylene ATP (Table 4). Suramin and pyridoxalphosphate-6-azophenyl-2',4'-disulfonic acid (PPADS) are examples of non-specific antagonists.

2. General composition information

a) Sequence similarities

It is understood that as discussed herein the use of the terms homology and identity mean the same thing as similarity. Thus, for example, if the use of the word homology is used between two non-natural sequences it is understood that this is not necessarily indicating an evolutionary relationship between these two sequences, but rather is looking at the similarity or relatedness between their nucleic acid sequences. Many of the methods for determining homology between two evolutionarily related molecules are routinely applied to any two or more nucleic acids or proteins for the purpose of measuring sequence similarity regardless of whether they are evolutionarily related or not.

For example, SEQ ID NO:1 represents a version of a P2X receptor. All fragments of the P2X receptor, as well as the other proteins, such as receptors discussed herein, are considered disclosed.

In general, it is understood that one way to define any known variants and derivatives or those that might arise, of the disclosed genes and proteins herein, is through defining the variants and derivatives in terms of homology to specific known sequences. This identity of particular sequences disclosed herein is also discussed elsewhere herein. In general, variants of genes and proteins herein disclosed typically have at least, about 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99 percent homology to the stated sequence or the native sequence. Those of skill in the art readily understand how to determine the homology of two proteins or nucleic acids, such as genes. For example, the homology can be calculated after aligning the two sequences so that the homology is at its highest level.

Another way of calculating homology can be performed by published algorithms. Optimal alignment of sequences for comparison can be conducted by the local homology algorithm of Smith and Waterman Adv. Appl. Math. 2: 482 (1981), by the homology alignment algorithm of Needleman and Wunsch, J. Mol. Biol. 48: 443 (1970), by the search for similarity method of Pearson and Lipman, Proc. Natl. Acad. Sci. U.S.A. 85: 2444 (1988), by computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group, 575 Science Dr., Madison, WI), or by inspection.

The same types of homology can be obtained for nucleic acids by for example the algorithms disclosed in Zuker, M. *Science* 244:48-52, 1989, Jaeger et al. *Proc. Natl. Acad. Sci. USA* 86:7706-7710, 1989, Jaeger et al. *Methods Enzymol.* 183:281-306, (1989) which are herein incorporated by reference for at least material related to nucleic acid alignment. It is understood that any of the methods typically can be used and that in certain instances the results of these various methods can differ, but the skilled artisan understands if identity is found with at least one of these methods, the sequences would be said to have the stated identity, and be disclosed herein.

For example, as used herein, a sequence recited as having a particular percent homology to another sequence refers to sequences that have the recited homology as calculated by any one or more of the calculation methods described above. For example, a first sequence has 80 percent homology, as defined herein, to a second sequence if the first sequence is calculated to have 80 percent homology to the second

sequence using the Zuker calculation method even if the first sequence does not have 80 percent homology to the second sequence as calculated by any of the other calculation methods. As another example, a first sequence has 80 percent homology, as defined herein, to a second sequence if the first sequence is calculated to have 80 percent homology to the second sequence using both the Zuker calculation method and the Pearson and Lipman calculation method even if the first sequence does not have 80 percent homology to the second sequence as calculated by the Smith and Waterman calculation method, the Needleman and Wunsch calculation method, the Jaeger calculation methods, or any of the other calculation methods. As yet another example, a first sequence has 80 percent homology, as defined herein, to a second sequence if the first sequence is calculated to have 80 percent homology to the second sequence using each of calculation methods (although, in practice, the different calculation methods will often result in different calculated homology percentages).

b) Hybridization

The term hybridization typically means a sequence driven interaction between at least two nucleic acid molecules, such as a primer or a probe and a gene. Sequence driven interaction means an interaction that occurs between two nucleotides or nucleotide analogs or nucleotide derivatives in a nucleotide specific manner. For example, G interacting with C or A interacting with T are sequence driven interactions. Typically sequence driven interactions occur on the Watson-Crick face or Hoogsteen face of the nucleotide. The hybridization of two nucleic acids is affected by a number of conditions and parameters known to those of skill in the art. For example, the salt concentrations, pH, and temperature of the reaction all affect whether two nucleic acid molecules will hybridize.

Parameters for selective hybridization between two nucleic acid molecules are well known to those of skill in the art. For example, in some embodiments selective hybridization conditions can be defined as stringent hybridization conditions. For example, stringency of hybridization is controlled by both temperature and salt concentration of either or both of the hybridization and washing steps. For example, the conditions of hybridization to achieve selective hybridization can involve hybridization in high ionic strength solution (6X SSC or 6X SSPE) at a temperature that is about 12-25°C below the Tm (the melting temperature at which half of the molecules dissociate from their hybridization partners) followed by washing at a combination of temperature and salt concentration chosen so that the washing temperature is about 5°C to 20°C below the Tm. The temperature and salt conditions are readily determined empirically in preliminary experiments in which samples of reference DNA immobilized on filters are hybridized to a labeled nucleic acid of interest and then washed under conditions of different stringencies. Hybridization temperatures are typically higher for DNA-RNA and RNA-RNA hybridizations. The conditions can be used as described above to achieve stringency, or as is known in the art. (Sambrook et al., Molecular Cloning: A Laboratory Manual, 2nd Ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, 1989; Kunkel et al. Methods Enzymol. 1987:154:367, 1987 which is herein incorporated by reference for material at least related to hybridization of nucleic acids). A preferable stringent hybridization condition for a DNA:DNA hybridization can be at about 68°C (in aqueous solution) in 6X SSC or 6X SSPE followed by washing at 68°C. Stringency of hybridization and washing, if desired, can be reduced accordingly as the degree of complementarity desired is decreased, and further,

depending upon the G-C or A-T richness of any area wherein variability is searched for. Likewise, stringency of hybridization and washing, if desired, can be increased accordingly as homology desired is increased, and further, depending upon the G-C or A-T richness of any area wherein high homology is desired, all as known in the art.

Another way to define selective hybridization is by looking at the amount (percentage) of one of the nucleic acids bound to the other nucleic acid. For example, in some embodiments selective hybridization conditions would be when at least about, 60, 65, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100 percent of the limiting nucleic acid is bound to the non-limiting nucleic acid. Typically, the non-limiting primer is in for example, 10 or 100 or 1000 fold excess. This type of assay can be performed at under conditions where both the limiting and non-limiting primer are for example, 10 fold or 100 fold or 1000 fold below their K_d, or where only one of the nucleic acid molecules is 10 fold or 100 fold or 1000 fold or where one or both nucleic acid molecules are above their K_d.

Another way to define selective hybridization is by looking at the percentage of primer that gets enzymatically manipulated under conditions where hybridization is required to promote the desired enzymatic manipulation. For example, in some embodiments selective hybridization conditions would be when at least about, 60, 65, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100 percent of the primer is enzymatically manipulated under conditions which promote the enzymatic manipulation, for example if the enzymatic manipulation is DNA extension, then selective hybridization conditions would be when at least about 60, 65, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100 percent of the primer molecules are extended. Preferred conditions also include those indicated by the manufacturer or indicated in the art as being appropriate for the enzyme performing the manipulation.

Just as with homology, it is understood that there are a variety of methods herein disclosed for determining the level of hybridization between two nucleic acid molecules. It is understood that these methods and conditions can provide different percentages of hybridization between two nucleic acid molecules, but unless otherwise indicated meeting the parameters of any of the methods would be sufficient. For example if 80% hybridization was required and as long as hybridization occurs within the required parameters in any one of these methods it is considered disclosed herein.

It is understood that those of skill in the art understand that if a composition or method meets any one of these criteria for determining hybridization either collectively or singly it is a composition or method that is disclosed herein.

c) Nucleic acids

There are a variety of molecules disclosed herein that are nucleic acid based, including for example the nucleic acids that encode, for example the purinergic receptors, as well as various functional nucleic acids. The disclosed nucleic acids are made up of, for example, nucleotides, nucleotide analogs, or nucleotide substitutes. Non-limiting examples of these and other molecules are discussed herein. It is understood that for example, when a vector is expressed in a cell that the expressed mRNA will typically be made up of A, C, G,

and U. Likewise, it is understood that if, for example, an antisense molecule is introduced into a cell or cell environment through for example exogenous delivery, it is advantageous that the antisense molecule be made up of nucleotide analogs that reduce the degradation of the antisense molecule in the cellular environment.

(1) Nucleotides and related molecules

A nucleotide is a molecule that contains a base moiety, a sugar moiety and a phosphate moiety. Nucleotides can be linked together through their phosphate moieties and sugar moieties creating an internucleoside linkage. The base moiety of a nucleotide can be adenin-9-yl (A), cytosin-1-yl (C), guanin-9-yl (G), uracil-1-yl (U), and thymin-1-yl (T). The sugar moiety of a nucleotide is a ribose or a deoxyribose. The phosphate moiety of a nucleotide is pentavalent phosphate. A non-limiting example of a nucleotide would be 3'-AMP (3'-adenosine monophosphate) or 5'-GMP (5'-guanosine monophosphate).

A nucleotide analog is a nucleotide, which contains some type of modification to the base, sugar, or phosphate moieties. Modifications to nucleotides are well known in the art and would include for example, 5-methylcytosine (5-me-C), 5-hydroxymethyl cytosine, xanthine, hypoxanthine, and 2-aminoadenine as well as modifications at the sugar or phosphate moieties.

Nucleotide substitutes are molecules having similar functional properties to nucleotides, but which do not contain a phosphate moiety, such as peptide nucleic acid (PNA). Nucleotide substitutes are molecules that will recognize nucleic acids in a Watson-Crick or Hoogsteen manner, but which are linked together through a moiety other than a phosphate moiety. Nucleotide substitutes are able to conform to a double helix type structure when interacting with the appropriate target nucleic acid.

It is also possible to link other types of molecules (conjugates) to nucleotides or nucleotide analogs to enhance for example, cellular uptake. Conjugates can be chemically linked to the nucleotide or nucleotide analogs. Such conjugates include but are not limited to lipid moieties such as a cholesterol moiety. (Letsinger et al., Proc. Natl. Acad. Sci. USA, 1989,86, 6553-6556),

A Watson-Crick interaction is at least one interaction with the Watson-Crick face of a nucleotide, nucleotide analog, or nucleotide substitute. The Watson-Crick face of a nucleotide, nucleotide analog, or nucleotide substitute includes the C2, N1, and C6 positions of a purine based nucleotide, nucleotide analog, or nucleotide substitute and the C2, N3, C4 positions of a pyrimidine based nucleotide, nucleotide analog, or nucleotide substitute.

A Hoogsteen interaction is the interaction that takes place on the Hoogsteen face of a nucleotide or nucleotide analog, which is exposed in the major groove of duplex DNA. The Hoogsteen face includes the N7 position and reactive groups (NH2 or O) at the C6 position of purine nucleotides.

(2) Nucleotide analogs and related molecules

A nucleotide analog is a nucleotide, which contains some type of modification to the base, sugar, or phosphate moieties. Modifications to the base moiety would include natural and synthetic modifications of A, C, G, and T/U as well as different purine or pyrimidine bases, such as uracil-5-yl (.psi.), hypoxanthin-9-yl (I), and 2-aminoadenin-9-yl. A modified base includes but is not limited to 5-methylcytosine (5-me-C), 5-hydroxymethyl cytosine, xanthine, hypoxanthine, 2-aminoadenine, 6-methyl and other alkyl derivatives of

adenine and guanine, 2-propyl and other alkyl derivatives of adenine and guanine, 2-thiouracil, 2-thiothymine and 2-thiocytosine, 5-halouracil and cytosine, 5-propynyl uracil and cytosine, 6-azo uracil, cytosine and thymine, 5-uracil (pseudouracil), 4-thiouracil, 8-halo, 8-amino, 8-thiol, 8-thioalkyl, 8-hydroxyl and other 8-substituted adenines and guanines, 5-halo particularly 5-bromo, 5-trifluoromethyl and other 5-substituted uracils and cytosines, 7-methylguanine and 7-methyladenine, 8-azaguanine and 8-azaadenine, 7-deazaguanine and 7-deazaadenine and 3-deazaguanine and 3-deazaadenine. Additional base modifications can be found for example in U.S. Pat. No. 3,687,808, Englisch et al., Angewandte Chemie, International Edition, 1991, 30, 613, and Sanghvi, Y. S., Chapter 15, Antisense Research and Applications, pages 289-302, Crooke, S. T. and Lebleu, B. ed., CRC Press, 1993. Certain nucleotide analogs, such as 5-substituted pyrimidines, 6-azapyrimidines and N-2, N-6 and O-6 substituted purines, including 2-aminopropyladenine, 5-propynyluracil and 5-propynylcytosine. 5-methylcytosine can increase the stability of duplex formation. Often time base modifications can be combined with for example a sugar modification, such as 2'-O-methoxyethyl, to achieve unique properties such as increased duplex stability. There are numerous United States patents such as 4,845,205; 5,130,302; 5,134,066; 5,175,273; 5,367,066; 5,432,272; 5,457,187; 5,459,255; 5,484,908; 5,502,177; 5,525,711; 5,552,540; 5,587,469; 5,594,121, 5,596,091; 5,614,617; and 5,681,941, which detail and describe a range of base modifications. Each of these patents is herein incorporated by reference.

Nucleotide analogs can also include modifications of the sugar moiety. Modifications to the sugar moiety would include natural modifications of the ribose and deoxy ribose as well as synthetic modifications. Sugar modifications include but are not limited to the following modifications at the 2' position: OH; F; O-, S-, or N-alkyl; O-, S-, or N-alkenyl; O-, S- or N-alkynyl; or O-alkyl-O-alkyl, wherein the alkyl, alkenyl and alkynyl can be substituted or unsubstituted C_1 to C_{10}, alkyl or C_2 to C_{10} alkenyl and alkynyl. 2' sugar modifications also include but are not limited to $-O[(CH_2)_n O]_m CH_3$, $-O(CH_2)_n OCH_3$, $-O(CH_2)_n NH_2$, $-O(CH_2)_n CH_3$, $-O(CH_2)_n -ONH_2$, and $-O(CH_2)_n ON[(CH_2)_n CH_3)]_2$, where n and m are from 1 to about 10.

Other modifications at the 2' position include but are not limited to: C_1 to C_{10} lower alkyl, substituted lower alkyl, alkaryl, aralkyl, O-alkaryl or O-aralkyl, SH, SCH_3, OCN, Cl, Br, CN, CF_3, OCF_3, $SOCH_3$, SO_2 CH_3, ONO_2, NO_2, N_3, NH_2, heterocycloalkyl, heterocycloalkaryl, aminoalkylamino, polyalkylamino, substituted silyl, an RNA cleaving group, a reporter group, an intercalator, a group for improving the pharmacokinetic properties of an oligonucleotide, or a group for improving the pharmacodynamic properties of an oligonucleotide, and other substituents having similar properties. Similar modifications can also be made at other positions on the sugar, particularly the 3' position of the sugar on the 3' terminal nucleotide or in 2'-5' linked oligonucleotides and the 5' position of 5' terminal nucleotide. Modified sugars would also include those that contain modifications at the bridging ring oxygen, such as CH_2 and S. Nucleotide sugar analogs can also have sugar mimetics such as cyclobutyl moieties in place of the pentofuranosyl sugar. There are numerous United States patents that teach the preparation of such modified sugar structures such as 4,981,957; 5,118,800; 5,319,080; 5,359,044; 5,393,878; 5,446,137; 5,466,786; 5,514,785; 5,519,134; 5,567,811; 5,576,427; 5,591,722; 5,597,909; 5,610,300; 5,627,053; 5,639,873; 5,646,265; 5,658,873; 5,670,633; and 5,700,920, each of which is herein incorporated by reference in its entirety.

Nucleotide analogs can also be modified at the phosphate moiety. Modified phosphate moieties include but are not limited to those that can be modified so that the linkage between two nucleotides contains a phosphorothioate, chiral phosphorothioate, phosphorodithioate, phosphotriester, aminoalkylphosphotriester, methyl and other alkyl phosphonates including 3'-alkylene phosphonate and chiral phosphonates, phosphinates, phosphoramidates including 3'-amino phosphoramidate and aminoalkylphosphoramidates, thionophosphoramidates, thionoalkylphosphonates, thionoalkylphosphotriesters, and boranophosphates. It is understood that these phosphate or modified phosphate linkage between two nucleotides can be through a 3'-5' linkage or a 2'-5' linkage, and the linkage can contain inverted polarity such as 3'-5' to 5'-3' or 2'-5' to 5'-2'. Various salts, mixed salts and free acid forms are also included. Numerous United States patents teach how to make and use nucleotides containing modified phosphates and include but are not limited to, 3,687,808; 4,469,863; 4,476,301; 5,023,243; 5,177,196; 5,188,897; 5,264,423; 5,276,019; 5,278,302; 5,286,717; 5,321,131; 5,399,676; 5,405,939; 5,453,496; 5,455,233; 5,466,677; 5,476,925; 5,519,126; 5,536,821; 5,541,306; 5,550,111; 5,563,253; 5,571,799; 5,587,361; and 5,625,050, each of which is herein incorporated by reference.

It is understood that nucleotide analogs need only contain a single modification, but can also contain multiple modifications within one of the moieties or between different moieties.

Nucleotide substitutes are molecules having similar functional properties to nucleotides, but which do not contain a phosphate moiety, such as peptide nucleic acid (PNA). Nucleotide substitutes are molecules that will recognize nucleic acids in a Watson-Crick or Hoogsteen manner, but which are linked together through a moiety other than a phosphate moiety. Nucleotide substitutes are able to conform to a double helix type structure when interacting with the appropriate target nucleic acid.

Nucleotide substitutes are nucleotides or nucleotide analogs that have had the phosphate moiety and/or sugar moieties replaced. Nucleotide substitutes do not contain a standard phosphorus atom. Substitutes for the phosphate can be for example, short chain alkyl or cycloalkyl internucleoside linkages, mixed heteroatom and alkyl or cycloalkyl internucleoside linkages, or one or more short chain heteroatomic or heterocyclic internucleoside linkages. These include those having morpholino linkages (formed in part from the sugar portion of a nucleoside); siloxane backbones; sulfide, sulfoxide and sulfone backbones, formacetyl and thioformacetyl backbones; methylene formacetyl and thioformacetyl backbones; alkene containing backbones; sulfamate backbones; methyleneimino and methylenehydrazino backbones; sulfonate and sulfonamide backbones; amide backbones; and others having mixed N, O, S and CH_2 component parts. Numerous United States patents disclose how to make and use these types of phosphate replacements and include but are not limited to 5,034,506; 5,166,315; 5,185,444; 5,214,134; 5,216,141; 5,235,033; 5,264,562; 5,264,564; 5,405,938; 5,434,257; 5,466,677; 5,470,967; 5,489,677; 5,541,307; 5,561,225; 5,596,086; 5,602,240; 5,610,289; 5,602,240; 5,608,046; 5,610,289; 5,618,704; 5,623,070; 5,663,312; 5,633,360; 5,677,437; and 5,677,439, each of which is herein incorporated by reference.

It is also understood in a nucleotide substitute that both the sugar and the phosphate moieties of the nucleotide can be replaced, by for example an amide type linkage (aminoethylglycine) (PNA). United States

patents 5,539,082; 5,714,331;and 5,719,262 teach how to make and use PNA molecules, each of which is herein incorporated by reference. (See also Nielsen et al., Science, 1991, 254, 1497-1500).

It is also possible to link other types of molecules (conjugates) to nucleotides or nucleotide analogs to enhance for example, cellular uptake. Conjugates can be chemically linked to the nucleotide or nucleotide analogs. Such conjugates include but are not limited to lipid moieties such as a cholesterol moiety (Letsinger et al., Proc. Natl. Acad. Sci. USA, 1989,86, 6553-6556), cholic acid (Manoharan et al., Bioorg. Med. Chem. Let., 1994, 4, 1053-1060), a thioether, e.g., hexyl-S-tritylthiol (Manoharan et al., Ann. N.Y. Acad. Sci., 1992, 660, 306-309; Manoharan et al., Bioorg. Med. Chem. Let., 1993, 3, 2765-2770), a thiocholesterol (Oberhauser et al., Nucl. Acids Res., 1992, 20, 533-538), an aliphatic chain, e.g., dodecandiol or undecyl residues (Saison-Behmoaras et al., EMBO J., 1991, 10, 1111-1118; Kabanov et al., FEBS Lett., 1990, 259, 327-330; Svinarchuk et al., Biochimie, 1993, 75, 49-54), a phospholipid, e.g., di-hexadecyl-rac-glycerol or triethylammonium 1,2-di-O-hexadecyl-rac-glycero-3-H-phosphonate (Manoharan et al., Tetrahedron Lett., 1995, 36, 3651-3654; Shea et al., Nucl. Acids Res., 1990, 18, 3777-3783), a polyamine or a polyethylene glycol chain (Manoharan et al., Nucleosides & Nucleotides, 1995, 14, 969-973), or adamantane acetic acid (Manoharan et al., Tetrahedron Lett., 1995, 36, 3651-3654), a palmityl moiety (Mishra et al., Biochim. Biophys. Acta, 1995, 1264, 229-237), or an octadecylamine or hexylamino-carbonyl-oxycholesterol moiety (Crooke et al., J. Pharmacol. Exp. Ther., 1996, 277, 923-937. Numerous United States patents teach the preparation of such conjugates and include, but are not limited to U.S. Pat. Nos. 4,828,979; 4,948,882; 5,218,105; 5,525,465; 5,541,313; 5,545,730; 5,552,538; 5,578,717, 5,580,731; 5,580,731; 5,591,584; 5,109,124; 5,118,802; 5,138,045; 5,414,077; 5,486,603; 5,512,439; 5,578,718; 5,608,046; 4,587,044; 4,605,735; 4,667,025; 4,762,779; 4,789,737; 4,824,941; 4,835,263; 4,876,335; 4,904,582; 4,958,013; 5,082,830; 5,112,963; 5,214,136; 5,082,830; 5,112,963; 5,214,136; 5,245,022; 5,254,469; 5,258,506; 5,262,536; 5,272,250; 5,292,873; 5,317,098; 5,371,241, 5,391,723; 5,416,203, 5,451,463; 5,510,475; 5,512,667; 5,514,785; 5,565,552; 5,567,810; 5,574,142; 5,585,481; 5,587,371; 5,595,726; 5,597,696; 5,599,923; 5,599,928 and 5,688,941, each of which is herein incorporated by reference.

A Watson-Crick interaction is at least one interaction with the Watson-Crick face of a nucleotide, nucleotide analog, or nucleotide substitute. The Watson-Crick face of a nucleotide, nucleotide analog, or nucleotide substitute includes the C2, N1, and C6 positions of a purine based nucleotide, nucleotide analog, or nucleotide substitute and the C2, N3, C4 positions of a pyrimidine based nucleotide, nucleotide analog, or nucleotide substitute.

A Hoogsteen interaction is the interaction that takes place on the Hoogsteen face of a nucleotide or nucleotide analog, which is exposed in the major groove of duplex DNA. The Hoogsteen face includes the N7 position and reactive groups (NH2 or O) at the C6 position of purine nucleotides.

(3) Sequences

There are a variety of sequences related to the purinergic receptors having the following Genbank Accession Numbers and these sequences and others are herein incorporated by reference in their entireties as well as for individual subsequences contained therein.

There are many sequences of the PX2 receptor, some of which can be found for example herein and others which can be found at Genbank, all of which are herein incorporated by reference. It is understood that the description related to this sequence is applicable to any sequence related to purinergic receptors, for example, unless specifically indicated otherwise. Those of skill in the art understand how to resolve sequence discrepancies and differences and to adjust the compositions and methods relating to a particular sequence to other related sequences. Primers and/or probes can be designed for any of the purinergic receptor sequences given the information disclosed herein and known in the art.

(4) Primers and probes

Disclosed are compositions including primers and probes, which are capable of interacting with the purinergic receptors as disclosed herein. In certain embodiments the primers are used to support DNA amplification reactions. Typically the primers will be capable of being extended in a sequence specific manner. Extension of a primer in a sequence specific manner includes any methods wherein the sequence and/or composition of the nucleic acid molecule to which the primer is hybridized or otherwise associated directs or influences the composition or sequence of the product produced by the extension of the primer. Extension of the primer in a sequence specific manner therefore includes, but is not limited to, PCR, DNA sequencing, DNA extension, DNA polymerization, RNA transcription, or reverse transcription. Techniques and conditions that amplify the primer in a sequence specific manner are preferred. In certain embodiments the primers are used for the DNA amplification reactions, such as PCR or direct sequencing. It is understood that in certain embodiments the primers can also be extended using non-enzymatic techniques, where for example, the nucleotides or oligonucleotides used to extend the primer are modified such that they will chemically react to extend the primer in a sequence specific manner. Typically the disclosed primers hybridize with a purinergic receptor nucleic acid or region of the purinergic receptor nucleic acid or they hybridize with the complement of the purinergic receptor nucleic acid or complement of a region of the purinergic receptor nucleic acid.

d) Delivery of the compositions to cells

(1) Nucleic Acid Delivery

There are a number of compositions and methods which can be used to deliver nucleic acids to cells, either in vitro or in vivo. These methods and compositions can largely be broken down into two classes: viral based delivery systems and non-viral based delivery systems. For example, the nucleic acids can be delivered through a number of direct delivery systems such as, electroporation, lipofection, calcium phosphate precipitation, plasmids, viral vectors, viral nucleic acids, phage nucleic acids, phages, cosmids, or via transfer of genetic material in cells or carriers such as cationic liposomes. Appropriate means for transfection, including viral vectors, chemical transfectants, or physico-mechanical methods such as electroporation and direct diffusion of DNA, are described by, for example, Wolff, J. A., et al., Science, 247, 1465-1468, (1990); and Wolff, J. A. Nature, 352, 815-818, (1991). Such methods are well known in the art and readily adaptable for use with the compositions and methods described herein. In certain cases, the methods will be modified to specifically function with large DNA molecules. Further, these methods can be used to target certain diseases and cell populations by using the targeting characteristics of the carrier.

In the methods described herein, which include the administration and uptake of exogenous DNA into the cells of a subject (i.e., gene transduction or transfection), the disclosed nucleic acids can be in the form of naked DNA or RNA, or the nucleic acids can be in a vector for delivering the nucleic acids to the cells, whereby the encoding DNA or DNA or fragment is under the transcriptional regulation of a promoter, as would be well understood by one of ordinary skill in the art as well as enhancers. The vector can be a commercially available preparation, such as an adenovirus vector (Quantum Biotechnologies, Inc. (Laval, Quebec, Canada).

As one example, vector delivery can be via a viral system, such as a retroviral vector system which can package a recombinant retroviral genome (see e.g., Pastan et al., *Proc. Natl. Acad. Sci. U.S.A.* 85:4486, 1988; Miller et al., *Mol. Cell. Biol.* 6:2895, 1986). The recombinant retrovirus can then be used to infect and thereby deliver to the infected cells nucleic acid encoding a broadly neutralizing antibody (or active fragment thereof). The exact method of introducing the altered nucleic acid into mammalian cells is, of course, not limited to the use of retroviral vectors. Other techniques are widely available for this procedure including the use of adenoviral vectors (Mitani et al., *Hum. Gene Ther.* 5:941-948, 1994), adeno-associated viral (AAV) vectors (Goodman et al., *Blood* 84:1492-1500, 1994), lentiviral vectors (Naidini et al., *Science* 272:263-267, 1996), pseudotyped retroviral vectors (Agrawal et al., *Exper. Hematol.* 24:738-747, 1996). Physical transduction techniques can also be used, such as liposome delivery and receptor-mediated and other endocytosis mechanisms (see, for example, Schwartzenberger et al., *Blood* 87:472-478, 1996). The disclosed compositions and methods can be used in conjunction with any of these or other commonly used gene transfer methods.

As one example, if the antibody-encoding nucleic acid or some other nucleic acid encoding a purinergic receptor interactions is delivered to the cells of a subject in an adenovirus vector, the dosage for administration of adenovirus to humans can range from about 10^7 to 10^9 plaque forming units (pfu) per injection but can be as high as 10^{12} pfu per injection (Crystal, *Hum. Gene Ther.* 8:985-1001, 1997; Alvarez and Curiel, *Hum. Gene Ther.* 8:597-613, 1997). A subject can receive a single injection, or, if additional injections are necessary, they can be repeated at six-month intervals (or other appropriate time intervals, as determined by the skilled practitioner) for an indefinite period and/or until the efficacy of the treatment has been established.

Parenteral administration of the nucleic acid or vector, if used, is generally characterized by injection. Injectables can be prepared in conventional forms, either as liquid solutions or suspensions, solid forms suitable for solution of suspension in liquid prior to injection, or as emulsions. A more recently revised approach for parenteral administration involves use of a slow release or sustained release system such that a constant dosage is maintained. See, e.g., U.S. Patent No. 3,610,795, which is incorporated by reference herein. For additional discussion of suitable formulations and various routes of administration of therapeutic compounds, see, e.g., *Remington: The Science and Practice of Pharmacy* (19th ed.) ed. A.R. Gennaro, Mack Publishing Company, Easton, PA 1995.

Nucleic acids that are delivered to cells which are to be integrated into the host cell genome, typically contain integration sequences. These sequences are often viral related sequences, particularly when viral

based systems are used. These viral integration systems can also be incorporated into nucleic acids which are to be delivered using a non-nucleic acid based system of deliver, such as a liposome, so that the nucleic acid contained in the delivery system can be come integrated into the host genome.

Other general techniques for integration into the host genome include, for example, systems designed to promote homologous recombination with the host genome. These systems typically rely on sequence flanking the nucleic acid to be expressed that has enough homology with a target sequence within the host cell genome that recombination between the vector nucleic acid and the target nucleic acid takes place, causing the delivered nucleic acid to be integrated into the host genome. These systems and the methods necessary to promote homologous recombination are known to those of skill in the art.

(2) Non-nucleic acid based systems

The disclosed compositions can be delivered to the target cells in a variety of ways. For example, the compositions can be delivered through electroporation, or through lipofection, or through calcium phosphate precipitation. The delivery mechanism chosen will depend in part on the type of cell targeted and whether the delivery is occurring for example in vivo or in vitro.

Thus, the compositions can comprise, in addition to the disclosed compositions or vectors for example, lipids such as liposomes, such as cationic liposomes (e.g., DOTMA, DOPE, DC-cholesterol) or anionic liposomes. Liposomes can further comprise proteins to facilitate targeting a particular cell, if desired. Administration of a composition comprising a compound and a cationic liposome can be administered to the blood afferent to a target organ or inhaled into the respiratory tract to target cells of the respiratory tract. Regarding liposomes, see, e.g., Brigham et al. *Am. J. Resp. Cell. Mol. Biol.* 1:95-100 (1989); Felgner et al. *Proc. Natl. Acad. Sci USA* 84:7413-7417 (1987); U.S. Pat. No.4,897,355. Furthermore, the compound can be administered as a component of a microcapsule that can be targeted to specific cell types, such as macrophages, or where the diffusion of the compound or delivery of the compound from the microcapsule is designed for a specific rate or dosage.

In the methods described above which include the administration and uptake of exogenous DNA into the cells of a subject (i.e., gene transduction or transfection), delivery of the compositions to cells can be via a variety of mechanisms. As one example, delivery can be via a liposome, using commercially available liposome preparations such as LIPOFECTIN, LIPOFECTAMINE (GIBCO-BRL, Inc., Gaithersburg, MD), SUPERFECT (Qiagen, Inc. Hilden, Germany) and TRANSFECTAM (Promega Biotec, Inc., Madison, WI), as well as other liposomes developed according to procedures standard in the art. In addition, the nucleic acid or vector can be delivered *in vivo* by electroporation, the technology for which is available from Genetronics, Inc. (San Diego, CA) as well as by means of a SONOPORATION machine (ImaRx Pharmaceutical Corp., Tucson, AZ).

The materials can be in solution, suspension (for example, incorporated into microparticles, liposomes, or cells). These can be targeted to a particular cell type via antibodies, receptors, or receptor ligands. The following references are examples of the use of this technology to target specific proteins to tumor tissue (Senter, et al., Bioconjugate Chem., 2:447-451, (1991); Bagshawe, K.D., Br. J. Cancer,

60:275-281, (1989); Bagshawe, et al., Br. J. Cancer, 58:700-703, (1988); Senter, et al., Bioconjugate Chem., 4:3-9, (1993); Battelli, et al., Cancer Immunol. Immunother., 35:421-425, (1992); Pietersz and McKenzie, Immunolog. Reviews, 129:57-80, (1992); and Roffler, et al., Biochem. Pharmacol, 42:2062-2065, (1991)). These techniques can be used for a variety of other specific cell types. Vehicles such as "stealth" and other antibody conjugated liposomes (including lipid mediated drug targeting to colonic carcinoma), receptor mediated targeting of DNA through cell specific ligands, lymphocyte directed tumor targeting, and highly specific therapeutic retroviral targeting of murine glioma cells *in vivo*. The following references are examples of the use of this technology to target specific proteins to tumor tissue (Hughes et al., Cancer Research, 49:6214-6220, (1989); and Litzinger and Huang, Biochimica et Biophysica Acta, 1104:179-187, (1992)). In general, receptors are involved in pathways of endocytosis, either constitutive or ligand induced. These receptors cluster in clathrin-coated pits, enter the cell via clathrin-coated vesicles, pass through an acidified endosome in which the receptors are sorted, and then either recycle to the cell surface, become stored intracellularly, or are degraded in lysosomes. The internalization pathways serve a variety of functions, such as nutrient uptake, removal of activated proteins, clearance of macromolecules, opportunistic entry of viruses and toxins, dissociation and degradation of ligand, and receptor-level regulation. Many receptors follow more than one intracellular pathway, depending on the cell type, receptor concentration, type of ligand, ligand valency, and ligand concentration. Molecular and cellular mechanisms of receptor-mediated endocytosis have been reviewed (Brown and Greene, DNA and Cell Biology 10:6, 399-409 (1991)).

(3) In vivo/ex vivo

As described above, the compositions can be administered in a pharmaceutically acceptable carrier and can be delivered to the subject's cells *in vivo* and/or *ex vivo* by a variety of mechanisms well known in the art (e.g., uptake of naked DNA, liposome fusion, intramuscular injection of DNA via a gene gun, endocytosis and the like).

If *ex vivo* methods are employed, cells or tissues can be removed and maintained outside the body according to standard protocols well known in the art. The compositions can be introduced into the cells via any gene transfer mechanism, such as, for example, calcium phosphate mediated gene delivery, electroporation, microinjection or proteoliposomes. The transduced cells can then be infused (e.g., in a pharmaceutically acceptable carrier) or homotopically transplanted back into the subject per standard methods for the cell or tissue type. Standard methods are known for transplantation or infusion of various cells into a subject.

e) Expression systems

The nucleic acids that are delivered to cells typically contain expression-controlling systems. For example, the inserted genes in viral and retroviral systems usually contain promoters, and/or enhancers to help control the expression of the desired gene product. A promoter is generally a sequence or sequences of DNA that function when in a relatively fixed location in regard to the transcription start site. A promoter contains core elements required for basic interaction of RNA polymerase and transcription factors, and can contain upstream elements and response elements.

(1) Viral Promoters and Enhancers

Preferred promoters controlling transcription from vectors in mammalian host cells can be obtained from various sources, for example, the genomes of viruses such as: polyoma, Simian Virus 40 (SV40), adenovirus, retroviruses, hepatitis-B virus and most preferably cytomegalovirus, or from heterologous mammalian promoters, e.g. beta actin promoter. The early and late promoters of the SV40 virus are conveniently obtained as an SV40 restriction fragment which also contains the SV40 viral origin of replication (Fiers et al., Nature, 273: 113 (1978)). The immediate early promoter of the human cytomegalovirus is conveniently obtained as a HindIII E restriction fragment (Greenway, P.J. et al., Gene 18: 355-360 (1982)). Of course, promoters from the host cell or related species also are useful herein.

Enhancer generally refers to a sequence of DNA that functions at no fixed distance from the transcription start site and can be either 5' (Laimins, L. et al., Proc. Natl. Acad. Sci. 78: 993 (1981)) or 3' (Lusky, M.L., et al., Mol.Cell Bio. 3:1108 (1983)) to the transcription unit. Furthermore, enhancers can be within an intron (Banerji, J.L. et al., Cell 33:729 (1983)) as well as within the coding sequence itself (Osborne, T.F., et al., Mol. Cell Bio. 4:1293 (1984)). They are usually between 10 and 300 bp in length, and they function in cis. Enhancers function to increase transcription from nearby promoters. Enhancers also often contain response elements that mediate the regulation of transcription. Promoters can also contain response elements that mediate the regulation of transcription. Enhancers often determine the regulation of expression of a gene. While many enhancer sequences are now known from mammalian genes (globin, elastase, albumin, -fetoprotein and insulin), typically one will use an enhancer from a eukaryotic cell virus for general expression. Examples are the SV40 enhancer on the late side of the replication origin (bp 100-270), the cytomegalovirus early promoter enhancer, the polyoma enhancer on the late side of the replication origin, and adenovirus enhancers.

The promoter and/or enhancer can be specifically activated either by light or specific chemical events which trigger their function. Systems can be regulated by reagents such as tetracycline and dexamethasone. There are also ways to enhance viral vector gene expression by exposure to irradiation, such as gamma irradiation, or alkylating chemotherapy drugs.

In certain embodiments the promoter and/or enhancer regions can act as a constitutive promoter and/or enhancer to maximize expression of the region of the transcription unit to be transcribed. In certain constructs the promoter and/or enhancer region be active in all eukaryotic cell types, even if it is only expressed in a particular type of cell at a particular time. A preferred promoter of this type is the CMV promoter (650 bases). Other preferred promoters are SV40 promoters, cytomegalovirus (full length promoter), and retroviral vector LTF.

It has been shown that all specific regulatory elements can be cloned and used to construct expression vectors that are selectively expressed in specific cell types such as melanoma cells. The glial fibrillary acetic protein (GFAP) promoter has been used to selectively express genes in cells of glial origin.

Expression vectors used in eukaryotic host cells (yeast, fungi, insect, plant, animal, human or nucleated cells) can also contain sequences necessary for the termination of transcription which can affect

mRNA expression. These regions are transcribed as polyadenylated segments in the untranslated portion of the mRNA encoding tissue factor protein. The 3' untranslated regions also include transcription termination sites. It is preferred that the transcription unit also contains a polyadenylation region. One benefit of this region is that it increases the likelihood that the transcribed unit will be processed and transported like mRNA. The identification and use of polyadenylation signals in expression constructs is well established. It is preferred that homologous polyadenylation signals be used in the transgene constructs. In certain transcription units, the polyadenylation region is derived from the SV40 early polyadenylation signal and consists of about 400 bases. It is also preferred that the transcribed units contain other standard sequences alone or in combination with the above sequences improve expression from, or stability of, the construct.

(2) Markers

The vectors can include nucleic acid sequence encoding a marker product. This marker product is used to determine if the gene has been delivered to the cell and once delivered is being expressed. Preferred marker genes are the *E. coli* lacZ gene, which encodes ß-galactosidase, and green fluorescent protein.

In some embodiments the marker can be a selectable marker. Examples of suitable selectable markers for mammalian cells are dihydrofolate reductase (DHFR), thymidine kinase, neomycin, neomycin analog G418, hydromycin, and puromycin. When such selectable markers are successfully transferred into a mammalian host cell, the transformed mammalian host cell can survive if placed under selective pressure. There are two widely used distinct categories of selective regimes. The first category is based on a cell's metabolism and the use of a mutant cell line which lacks the ability to grow independent of a supplemented media. Two examples are: CHO DHFR- cells and mouse LTK- cells. These cells lack the ability to grow without the addition of such nutrients as thymidine or hypoxanthine. Because these cells lack certain genes necessary for a complete nucleotide synthesis pathway, they cannot survive unless the missing nucleotides are provided in a supplemented media. An alternative to supplementing the media is to introduce an intact DHFR or TK gene into cells lacking the respective genes, thus altering their growth requirements. Individual cells which were not transformed with the DHFR or TK gene will not be capable of survival in non-supplemented media.

The second category is dominant selection which refers to a selection scheme used in any cell type and does not require the use of a mutant cell line. These schemes typically use a drug to arrest growth of a host cell. Those cells which have a novel gene would express a protein conveying drug resistance and would survive the selection. Examples of such dominant selection use the drugs neomycin, (Southern P. and Berg, P., J. Molec. Appl. Genet. 1:327 (1982)), mycophenolic acid, (Mulligan, R.C. and Berg, P. Science 209:1422 (1980)) or hygromycin, (Sugden, B. et al., Mol. Cell. Biol. 5:410-413 (1985)). The three examples employ bacterial genes under eukaryotic control to convey resistance to the appropriate drug G418 or neomycin (geneticin), xgpt (mycophenolic acid) or hygromycin, respectively. Others include the neomycin analog G418 and puromycin.

f) Peptides

(1) Protein variants

As discussed herein there are numerous variants of the purinergic receptor proteins and that are known and herein contemplated. In addition, to the known functional purinergic receptor species variants there are derivatives of the purinergic receptor proteins which also function in the disclosed methods and compositions. Protein variants and derivatives are well understood to those of skill in the art and in can involve amino acid sequence modifications. For example, amino acid sequence modifications typically fall into one or more of three classes: substitutional, insertional or deletional variants. Insertions include amino and/or carboxyl terminal fusions as well as intrasequence insertions of single or multiple amino acid residues. Insertions ordinarily will be smaller insertions than those of amino or carboxyl terminal fusions, for example, on the order of one to four residues. Immunogenic fusion protein derivatives, such as those described in the examples, are made by fusing a polypeptide sufficiently large to confer immunogenicity to the target sequence by cross-linking in vitro or by recombinant cell culture transformed with DNA encoding the fusion. Deletions are characterized by the removal of one or more amino acid residues from the protein sequence. Typically, no more than about from 2 to 6 residues are deleted at any one site within the protein molecule. These variants ordinarily are prepared by site-specific mutagenesis of nucleotides in the DNA encoding the protein, thereby producing DNA encoding the variant, and thereafter expressing the DNA in recombinant cell culture. Techniques for making substitution mutations at predetermined sites in DNA having a known sequence are well known, for example M13 primer mutagenesis and PCR mutagenesis. Amino acid substitutions are typically of single residues, but can occur at a number of different locations at once; insertions usually will be on the order of about from 1 to 10 amino acid residues; and deletions will range about from 1 to 30 residues. Deletions or insertions preferably are made in adjacent pairs, i.e. a deletion of 2 residues or insertion of 2 residues. Substitutions, deletions, insertions or any combination thereof can be combined to arrive at a final construct. The mutations must not place the sequence out of reading frame and preferably will not create complementary regions that could produce secondary mRNA structure. Substitutional variants are those in which at least one residue has been removed and a different residue inserted in its place. Such substitutions generally are made in accordance with the following Tables 5 and 6 and are referred to as conservative substitutions.

TABLE 5:Amino Acid Abbreviations

Amino Acid	Abbreviations
Alanine	AlaA
Allosoleucine	Aile
Arginine	ArgR
Asparagines	AsnN
Aspartic acid	AspD
Cysteine	CysC
Glutamic acid	GluE
Glutamine	GlnQ
Glycine	GlyG
Histidine	HisH
Isolelucine	IleI
Leucine	LeuL

Amino Acid	Abbreviations
Lysine	LysK
Phenylalanine	PheF
Proline	ProP
Pyroglutamic acid	Glu
Serine	SerS
Threonine	ThrT
Tyrosine	TyrY
Tryptophan	TrpW
Valine	ValV

Table 6.

Original Residue Exemplary Conservative Substitutions, others are known in the art.

Ala	ser
Arg	lys, gln
Asn	gln; his
Asp	glu
Cys	ser
Gln	asn, lys
Glu	asp
Gly	ala
His	asn;gln
Ile	leu; val
Leu	ile; val
Lys	arg; gln;
Met	Leu; ile
Phe	met; leu; tyr
Ser	thr
Thr	ser
Trp	tyr
Tyr	trp; phe
Val	ile; leu

Substantial changes in function or immunological identity are made by selecting substitutions that are less conservative than those in Table 6, i.e., selecting residues that differ more significantly in their effect on maintaining (a) the structure of the polypeptide backbone in the area of the substitution, for example as a sheet or helical conformation, (b) the charge or hydrophobicity of the molecule at the target site or (c) the bulk of the side chain. The substitutions which in general are expected to produce the greatest changes in the protein properties will be those in which (a) a hydrophilic residue, e.g. seryl or threonyl, is substituted for (or by) a hydrophobic residue, e.g. leucyl, isoleucyl, phenylalanyl, valyl or alanyl; (b) a cysteine or proline is substituted for (or by) any other residue; (c) a residue having an electropositive side chain, e.g., lysyl, arginyl, or histidyl, is substituted for (or by) an electronegative residue, e.g., glutamyl or aspartyl; or (d) a residue having a bulky side chain, e.g., phenylalanine, is substituted for (or by) one not having a side chain, e.g., glycine, in this case, (e) by increasing the number of sites for sulfation and/or glycosylation.

For example, the replacement of one amino acid residue with another that is biologically and/or chemically similar is known to those skilled in the art as a conservative substitution. For example, a conservative substitution would be replacing one hydrophobic residue for another, or one polar residue for another. The substitutions include combinations such as, for example, Gly, Ala; Val, Ile, Leu; Asp, Glu; Asn, Gln; Ser, Thr; Lys, Arg; and Phe, Tyr. Such conservatively substituted variations of each explicitly disclosed sequence are included within the mosaic polypeptides provided herein.

Substitutional or deletional mutagenesis can be employed to insert sites for N-glycosylation (Asn-X-Thr/Ser) or O-glycosylation (Ser or Thr). Deletions of cysteine or other labile residues also can be desirable. Deletions or substitutions of potential proteolysis sites, e.g. Arg, is accomplished for example by deleting one of the basic residues or substituting one by glutaminyl or histidyl residues.

Certain post-translational derivatizations are the result of the action of recombinant host cells on the expressed polypeptide. Glutaminyl and asparaginyl residues are frequently post-translationally deamidated to the corresponding glutamyl and asparyl residues. Alternatively, these residues are deamidated under mildly acidic conditions. Other post-translational modifications include hydroxylation of proline and lysine, phosphorylation of hydroxyl groups of seryl or threonyl residues, methylation of the o-amino groups of lysine, arginine, and histidine side chains (T.E. Creighton, Proteins: Structure and Molecular Properties, W. H. Freeman & Co., San Francisco pp 79-86 [1983]), acetylation of the N-terminal amine and, in some instances, amidation of the C-terminal carboxyl.

It is understood that one way to define the variants and derivatives of the disclosed proteins herein is through defining the variants and derivatives in terms of homology/identity to specific known sequences. For example, SEQ ID NO:1 sets forth a particular sequence of a P2X receptor. Specifically disclosed are variants of these and other proteins herein disclosed which have at least, 70% or 75% or 80% or 85% or 90% or 95% homology to the stated sequence. Those of skill in the art readily understand how to determine the homology of two proteins. For example, the homology can be calculated after aligning the two sequences so that the homology is at its highest level.

Another way of calculating homology can be performed by published algorithms. Optimal alignment of sequences for comparison can be conducted by the local homology algorithm of Smith and Waterman Adv. Appl. Math. 2: 482 (1981), by the homology alignment algorithm of Needleman and Wunsch, J. Mol. Biol. 48: 443 (1970), by the search for similarity method of Pearson and Lipman, Proc. Natl. Acad. Sci. U.S.A. 85: 2444 (1988), by computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group, 575 Science Dr., Madison, WI), or by inspection.

The same types of homology can be obtained for nucleic acids by for example the algorithms disclosed in Zuker, M. *Science* 244:48-52, 1989, Jaeger et al. *Proc. Natl. Acad. Sci. USA* 86:7706-7710, 1989, Jaeger et al. *Methods Enzymol.* 183:281-306, 1989 which are herein incorporated by reference for at least material related to nucleic acid alignment.

It is understood that the description of conservative mutations and homology can be combined together in any combination, such as embodiments that have at least 70% homology to a particular sequence wherein the variants are conservative mutations.

As this specification discusses various proteins and protein sequences it is understood that the nucleic acids that can encode those protein sequences are also disclosed. This would include all degenerate sequences related to a specific protein sequence, i.e. all nucleic acids having a sequence that encodes one particular protein sequence as well as all nucleic acids, including degenerate nucleic acids, encoding the disclosed

variants and derivatives of the protein sequences. Thus, while each particular nucleic acid sequence cannot be written out herein, it is understood that each and every sequence is in fact disclosed and described herein through the disclosed protein sequence. It is also understood that while no amino acid sequence indicates what particular DNA sequence encodes that protein within an organism, where particular variants of a disclosed protein are disclosed herein, the known nucleic acid sequence that encodes that protein in the particular organism from which that protein arises is also known and herein disclosed and described.

g) Pharmaceutical carriers/Delivery of pharmaceutical products

As described herein, the compositions, such as the ATP and ATP analogs, can also be administered *in vivo* in a pharmaceutically acceptable carrier. By "pharmaceutically acceptable" is meant a material that is not biologically or otherwise undesirable, i.e., the material can be administered to a subject, along with the nucleic acid or vector, without causing any undesirable biological effects or interacting in a deleterious manner with any of the other components of the pharmaceutical composition in which it is contained. The carrier would naturally be selected to minimize any degradation of the active ingredient and to minimize any adverse side effects in the subject, as would be well known to one of skill in the art.

The compositions can be administered orally, parenterally (e.g., intravenously), by intramuscular injection, by intraperitoneal injection, transdermally, extracorporeally, topically or the like, including topical intranasal administration or administration by inhalant. As used herein, "topical intranasal administration" means delivery of the compositions into the nose and nasal passages through one or both of the nares and can comprise delivery by a spraying mechanism or droplet mechanism, or through aerosolization of the composition. Administration of the compositions by inhalant can be through the nose or mouth via delivery by a spraying or droplet mechanism. Delivery can also be directly to any area of the respiratory system (e.g., lungs) via intubation. The exact amount of the compositions required will vary from subject to subject, depending on the species, age, weight and general condition of the subject, the severity of the allergic disorder being treated, the particular nucleic acid or vector used, its mode of administration and the like. Thus, it is not possible to specify an exact amount for every composition. However, an appropriate amount can be determined by one of ordinary skill in the art using only routine experimentation given the teachings herein.

Parenteral administration of the composition, if used, is generally characterized by injection. Injectables can be prepared in conventional forms, either as liquid solutions or suspensions, solid forms suitable for solution of suspension in liquid prior to injection, or as emulsions. A more recently revised approach for parenteral administration involves use of a slow release or sustained release system such that a constant dosage is maintained. See, e.g., U.S. Patent No. 3,610,795, which is incorporated by reference herein.

The materials can be in solution, suspension (for example, incorporated into microparticles, liposomes, or cells). These can be targeted to a particular cell type via antibodies, receptors, or receptor ligands. The following references are examples of the use of this technology to target specific proteins to tumor tissue (Senter, et al., Bioconjugate Chem., 2:447-451, (1991); Bagshawe, K.D., Br. J. Cancer, 60:275-281, (1989); Bagshawe, et al., Br. J. Cancer, 58:700-703, (1988); Senter, et al., Bioconjugate Chem., 4:3-9, (1993); Battelli, et al., Cancer Immunol. Immunother., 35:421-425, (1992); Pietersz and McKenzie,

Immunolog. Reviews, 129:57-80, (1992); and Roffler, et al., Biochem. Pharmacol, 42:2062-2065, (1991)). Vehicles such as "stealth" and other antibody conjugated liposomes (including lipid mediated drug targeting to colonic carcinoma), receptor mediated targeting of DNA through cell specific ligands, lymphocyte directed tumor targeting, and highly specific therapeutic retroviral targeting of murine glioma cells *in vivo*. The following references are examples of the use of this technology to target specific proteins to tumor tissue (Hughes et al., Cancer Research, 49:6214-6220, (1989); and Litzinger and Huang, Biochimica et Biophysica Acta, 1104:179-187, (1992)). In general, receptors are involved in pathways of endocytosis, either constitutive or ligand induced. These receptors cluster in clathrin-coated pits, enter the cell via clathrin-coated vesicles, pass through an acidified endosome in which the receptors are sorted, and then either recycle to the cell surface, become stored intracellularly, or are degraded in lysosomes. The internalization pathways serve a variety of functions, such as nutrient uptake, removal of activated proteins, clearance of macromolecules, opportunistic entry of viruses and toxins, dissociation and degradation of ligand, and receptor-level regulation. Many receptors follow more than one intracellular pathway, depending on the cell type, receptor concentration, type of ligand, ligand valency, and ligand concentration. Molecular and cellular mechanisms of receptor-mediated endocytosis have been reviewed (Brown and Greene, DNA and Cell Biology 10:6, 399-409 (1991)).

(1) Pharmaceutically Acceptable Carriers

Suitable carriers and their formulations are described in *Remington: The Science and Practice of Pharmacy* (19th ed.) ed. A.R. Gennaro, Mack Publishing Company, Easton, PA 1995. Typically, an appropriate amount of a pharmaceutically-acceptable salt is used in the formulation to render the formulation isotonic. Examples of the pharmaceutically-acceptable carrier include, but are not limited to, saline, Ringer's solution and dextrose solution. The pH of the solution is preferably from about 5 to about 8, and more preferably from about 7 to about 7.5. Further carriers include sustained release preparations such as semipermeable matrices of solid hydrophobic polymers containing the antibody, which matrices are in the form of shaped articles, e.g., films, liposomes or microparticles. It will be apparent to those persons skilled in the art that certain carriers can be more preferable depending upon, for instance, the route of administration and concentration of composition being administered.

Pharmaceutical carriers are known to those skilled in the art. These most typically would be standard carriers for administration of drugs to humans, including solutions such as sterile water, saline, and buffered solutions at physiological pH. The compositions can be administered intramuscularly or subcutaneously. Other compounds will be administered according to standard procedures used by those skilled in the art.

Pharmaceutical compositions can include carriers, thickeners, diluents, buffers, preservatives, surface-active agents and the like in addition to the molecule of choice. Pharmaceutical compositions can also include one or more active ingredients such as antimicrobial agents, antiinflammatory agents, anesthetics, and the like.

The pharmaceutical composition can be administered in a number of ways depending on whether local or systemic treatment is desired, and on the area to be treated. Administration can be topically (including ophthalmically, vaginally, rectally, intranasally), orally, by inhalation, or parenterally, for example by intravenous

drip, subcutaneous, intraperitoneal or intramuscular injection. The disclosed antibodies can be administered intravenously, intraperitoneally, intramuscularly, subcutaneously, intracavity, or transdermally.

Preparations for parenteral administration include sterile aqueous or non-aqueous solutions, suspensions, and emulsions. Examples of non-aqueous solvents are propylene glycol, polyethylene glycol, vegetable oils such as olive oil, and injectable organic esters such as ethyl oleate. Aqueous carriers include water, alcoholic/aqueous solutions, emulsions or suspensions, including saline and buffered media. Parenteral vehicles include sodium chloride solution, Ringer's dextrose, dextrose and sodium chloride, lactated Ringer's, or fixed oils. Intravenous vehicles include fluid and nutrient replenishers, electrolyte replenishers (such as those based on Ringer's dextrose), and the like. Preservatives and other additives can also be present such as, for example, antimicrobials, anti-oxidants, chelating agents, and inert gases and the like.

Formulations for topical administration can include ointments, lotions, creams, gels, drops, suppositories, sprays, liquids and powders. Conventional pharmaceutical carriers, aqueous, powder or oily bases, thickeners and the like can be necessary or desirable.

Compositions for oral administration include powders or granules, suspensions or solutions in water or non-aqueous media, capsules, sachets, or tablets. Thickeners, flavorings, diluents, emulsifiers, dispersing aids or binders can be desirable.

Some of the compositions can potentially be administered as a pharmaceutically acceptable acid- or base- addition salt, formed by reaction with inorganic acids such as hydrochloric acid, hydrobromic acid, perchloric acid, nitric acid, thiocyanic acid, sulfuric acid, and phosphoric acid, and organic acids such as formic acid, acetic acid, propionic acid, glycolic acid, lactic acid, pyruvic acid, oxalic acid, malonic acid, succinic acid, maleic acid, and fumaric acid, or by reaction with an inorganic base such as sodium hydroxide, ammonium hydroxide, potassium hydroxide, and organic bases such as mono-, di-, trialkyl and aryl amines and substituted ethanolamines.

(2) Therapeutic Uses

Effective dosages and schedules for administering the compositions can be determined empirically, and making such determinations is within the skill in the art. The dosage ranges for the administration of the compositions are those large enough to produce the desired effect in which the symptoms of the disorder are affected. The dosage should not be so large as to cause adverse side effects, such as unwanted cross-reactions, anaphylactic reactions, and the like. Generally, the dosage will vary with the age, condition, sex and extent of the disease in the patient, route of administration, or whether other drugs are included in the regimen, and can be determined by one of skill in the art. The dosage can be adjusted by the individual physician in the event of any counterindications. Dosage can vary, and can be administered in one or more dose administrations hourly or daily,for one or several days. Guidance can be found in the literature for appropriate dosages for given classes of pharmaceutical products. For example, guidance in selecting appropriate doses for antibodies can be found in the literature on therapeutic uses of antibodies, e.g., Handbook of Monoclonal Antibodies, Ferrone et al., eds., Noges Publications, Park Ridge, N.J., (1985) ch. 22 and pp. 303-357; Smith et al., Antibodies in Human Diagnosis and Therapy, Haber et al., eds., Raven Press, New York (1977) pp. 365-389. A typical

daily dosage of the ATP or ATP analogs used alone might range from about 1 μg/kg to up to 100 mg/kg of body weight or more per day, depending on the factors mentioned above. For example, based on the similarities of EC_{50} concentrations for P2 receptors across many species an effective dose to modulate smell in a human would be similar to our mouse model, i.e., 10-200 μM.

Following administration of a disclosed composition, such as an ATP analog, for the modulation of smell, the efficacy of the therapeutic composition can be assessed in various ways well known to the skilled practitioner. For instance, one of ordinary skill in the art will understand that the compositions disclosed herein are efficacious in modulating, such as enhancing or reducing the sensation of smell in a subject, by observing that the composition reduces or enhances the sensation of smell to a particular or general odor stimulant. Smell sensation can be measured by methods that are known in the art, for example, and in vitro methods using an ORN calcium imaging assay as discussed herein, can also be used.

The compositions that modulate smell disclosed herein can be administered prophylactically to patients or subjects who are at risk for being exposed to severe or damaging odor stimulation or who have a desire to have either an increased or decreased sensitivity to an odor. For example, elderly people can increase sensitivity to odor to compensate for loss during aging. Conversely, those on chemotherapy drugs may need decreased odor sensitivity to reduce nausea.

Other molecules that modulate odor sensitivity, but do not have a particular pharmaceutical function can be used for tracking changes within ORNs.

h) Computer readable mediums

It is understood that the disclosed nucleic acids and proteins and compositions can be represented as a sequence consisting of the nucleotides of amino acids. There are a variety of ways to display these sequences, for example the nucleotide guanosine can be represented by G or g. Likewise the amino acid valine can be represented by Val or V. Those of skill in the art understand how to display and express any nucleic acid or protein sequence in any of the variety of ways that exist, each of which is considered herein disclosed. Specifically contemplated herein is the display of these sequences and ATP or ATP analogs on computer readable mediums, such as, commercially available floppy disks, tapes, chips, hard drives, compact disks, and videodisks, or other computer readable mediums. Also disclosed are the binary code representations of the disclosed sequences and ATP or ATP analogs. Those of skill in the art understand what computer readable mediums. Thus, computer readable mediums on which the nucleic acids or protein sequences are recorded, stored, or saved

i) Chips and micro arrays

Disclosed are chips where at least one address is the sequences or part of the sequences set forth in any of the nucleic acid sequences disclosed herein. Also disclosed are chips where at least one address is the sequences or portion of sequences set forth in any of the peptide sequences disclosed herein. Disclosed are chips where at least one address is the composition, such as an ATP analog, disclosed herein.

Also disclosed are chips where at least one address is a variant of the sequences or part of the sequences set forth in any of the nucleic acid sequences disclosed herein. Also disclosed are chips where at

least one address is a variant of the sequences or portion of sequences set forth in any of the peptide sequences disclosed herein.

j) Kits

Disclosed herein are kits that are drawn to reagents that can be used in practicing the methods disclosed herein. The kits can include any reagent or combination of reagent discussed herein or that would be understood to be required or beneficial in the practice of the disclosed methods. For example, the kits could include an ATP analog in a formulation for delivery to an ORN. For example, disclosed is a kit for modulating odor sensitivity comprising ATP in a formulation for delivery to an ORN.

D. Methods of making the compositions

The compositions disclosed herein and the compositions necessary to perform the disclosed methods can be made using any method known to those of skill in the art for that particular reagent or compound unless otherwise specifically noted.

1. ATP analog generation

The disclosed ATP analogs can be made using a variety of synthetic procedures. Often the analogs can be purchased. For example, the following analogs can be purchased from Sigma Inc., 2-(Methylthio)adenosine 5'-triphosphate, 2-Chloroadenosine 5' triphosphate, 2',5'-Dideoxyadenosine 3'-triphosphate, 2'-3'-O-(4-Benzoylbenzoyl)adenosine 5'-triphosphate, 2'-Monophosphoadenosine 5'-diphosphoribose, ATP-Ribose, 8-Bromoadenosine 5'-triphosphate, Adenosine 5'-triphosphate P3-[1-(2-nitrophenyl)ethyl ester] (Caged ATP'NPE caged ATP), Adenosine 5'-triphosphate, Adenosine 5'-(β,γ-imido)triphosphate, Adenosine 5'-[γ-thio]triphosphate, Adenosine 5'-triphosphate g-(1-[2-nitrophenyl]ethyl) ester (Caged ATP), 125229-58-5minimum 95%, and 2',3'-O-(2,4,6-Trinitrophenyl) adenosine 5'-triphosphate. It is understood that these compositions and others come as salts, such as lithium, or sodium salts, as well as, for example, triethylammonium salts, and that they can also be formulated in appropriate pharmaceutical salts.

2. Nucleic acid synthesis

For example, the nucleic acids, such as, the oligonucleotides to be used as primers can be made using standard chemical synthesis methods or can be produced using enzymatic methods or any other known method. Such methods can range from standard enzymatic digestion followed by nucleotide fragment isolation (see for example, Sambrook *et al.*, *Molecular Cloning: A Laboratory Manual*, 2nd Edition (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1989) Chapters 5, 6) to purely synthetic methods, for example, by the cyanoethyl phosphoramidite method using a Milligen or Beckman System 1Plus DNA synthesizer (for example, Model 8700 automated synthesizer of Milligen-Biosearch, Burlington, MA or ABI Model 380B). Synthetic methods useful for making oligonucleotides are also described by Ikuta *et al.*, *Ann. Rev. Biochem.* 53:323-356 (1984), (phosphotriester and phosphite-triester methods), and Narang *et al.*, *Methods Enzymol.*, 65:610-620 (1980), (phosphotriester method). Protein nucleic acid molecules can be made using known methods such as those described by Nielsen *et al.*, *Bioconjug. Chem.* 5:3-7 (1994).

E. Methods of using the compositions

1. Methods of using the compositions as research tools

The disclosed compositions can be used in a variety of ways as research tools. For example, the disclosed compositions, such as ATP and analogs, can be used to study the signal transduction pathways related to olfactory signaling.

The disclosed compositions can be used as discussed herein as either reagents in micro arrays or as reagents to probe or analyze existing microarrays. The compositions can also be used in any known method of screening assays, related to chip/micro arrays. The compositions can also be used in any known way of using the computer readable embodiments of the disclosed compositions, for example, to study relatedness or to perform molecular modeling analysis related to the disclosed compositions.

a) Screening assays

Provided are methods of screening for an agonist or an antagonist of purinergic receptor of the olfactory system, comprising contacting a purinergic receptor with a test compound; detecting intracellular calcium levels; and screening for a change in calcium levels as compared to a control level, a change indicating the compound is an agonist or an antagonist of the olfactory system.

Screening optionally takes place in multi-well plates. Multi-well plates are standard in the art and come in a variety of sizes and shapes. For example, the multi-well plate can be 24, 48, or 96 well plates. Such screening assays can be automated or further modified for high throughput analysis. For high throughput screening, each well can include numerous test components. If a positive reaction is detected in a well, the screening is repeated with one of the test compounds contained in a single well.

An "elevation in calcium" is defined as an increase in calcium levels greater than 1 nM above basal levels. The change in calcium levels can be between 5 nM and 10 nM, for example. The elevation in calcium can also be greater than 100 nM above basal levels. A "transient reduction in calcium" is defined as decrease in calcium levels greater than 1 nM below basal levels. The reduction in calcium can also be greater than 100 nM below basal levels.

The time defined as "transient" means not permanent. Thus, transient can be less than 10 seconds, less than 30 seconds, less than 1 minute, less than 5 minutes, less than 10 minutes, or less than 20 minutes, for example.

The term "sustained" means that the effect continues for a period of tim. For example, sustained can be greater than 1 minute, greater than 5 minutes, greater than 10 minutes, greater than an hour, greater than 24 hours, or greater than 1 year.

Also disclosed is a method of screening for an agonist or an antagonist of a purinergic receptor of the olfactory system, comprising contacting a first purinergic receptor expressing cell with a set of test compounds; detecting calcium levels in the first purinergic receptor cell; and selecting each compound in the set that contacted the first purinergic receptor cell, wherein the first purinergic receptor cell showed a transient change in calcium as compared to a control level, indicating the compound is an agonist or an antagonist of a purinergic receptor of the olfactory system. The method can further comprise the steps of contacting a second

purinergic receptor cell with one test compound selected above, and detecting calcium levels in the second purinergic receptor cell, wherein a transient change in calcium as compared to a control level indicates the compound is an agonist or an antagonist of a purinergic receptor of the olfactory system.

Also disclosed is a method of screening for an agonist or an antagonist of a purinergic receptor of the olfactory system, comprising contacting a test compound with a cell that expresses a heterologous nucleic acid that encodes a purinergic receptor; and detecting calcium levels in the cell; a transient change in calcium as compared to a control level, indicating an agonist or an antagonist of a purinergic receptor of the olfactory system..

Also contemplated are agents identified by the screening methods described herein, as well as methods of making those agents. An example of a method of making an agent includes identifying the agent using the methods provided herein, and manufacturing the agent or manufacturing the agent in a pharmaceutically acceptable carrier.

Preferably, the cell is a cell that lacks the receptor prior to introduction of the heterologous nucleic acid. The cell can be transiently transfected with the heterologous nucleic acid or a stable cell line containing the expressed receptor can be made using standard techniques in the art. By "heterologous nucleic acid" is meant that any heterologous or exogenous nucleic acid can be inserted into a vector for transfer into a cell, tissue or organism. The nucleic acid can encode a polypeptide or protein or an antisense RNA, for example. The nucleic acid can be functionally linked to a promoter. By "functionally linked" is meant such that the promoter can promote expression of the heterologous nucleic acid, as is known in the art, such as appropriate orientation of the promoter relative to the heterologous nucleic acid. Furthermore, the heterologous nucleic acid preferably has all appropriate sequences for expression of the nucleic acid, as known in the art, to functionally encode, i.e., allow the nucleic acid to be expressed. The nucleic acid can include, for example, expression control sequences, such as an enhancer, and necessary information processing sites, such as ribosome binding sites, RNA splice sites, polyadenylation sites, and transcriptional terminator sequences.

Calcium levels and changes in calcium levels can be detected using a calcium indicator such as the cell-permeable methyl ester form of Fura-2, which is Fura-2/AM. In another example, a fluorescence plate reader is used that detects a single wavelength, such as Ca^{2+} indicator dyes Fluo 3, Fluo 4 AM, Quin 2, Indo-1 and Indo-4.

Optionally, the compound being screened can augment the effects of other compounds such as ATP, for example. In this case, the compound being screened can be tested in the presence of another compound that stimulates the purinergic receptor. For example, the purinergic receptor expressing cell can be in a solution containing an effective amount of ATP. An "effective amount of ATP" is defined as about 300 nM to about 1 mM of ATP.

F. Examples

Efforts have been made to ensure accuracy with respect to numbers (e.g., amounts, temperature, etc.), but some errors and deviations should be accounted for. Unless indicated otherwise, parts are parts by weight, temperature is in °C or is at ambient temperature, and pressure is at or near atmospheric.

1. Example 1 ATP differentially modulates odor responsiveness through purinergic receptor activation: activation of purinergic receptor subtypes differentially modulates odor sensitivity

a) Results

(1) Localization of Purinergic Receptors in the Peripheral Olfactory System

Using RT-PCR and immunohistochemical methods ionotropic $P2X_2$ and G-protein-coupled $P2Y_2$ receptor expression were found in both olfactory epithelium and olfactory bulb. RT-PCR analysis revealed mRNA expression for the $P2Y_2$ receptor and two isoforms of the $P2X_2$ receptor; $P2X_{2\text{-}1}$ (Brake, A. J., et al., Nature 371, 519-523 (1994)) and $P2X_{2\text{-}2}$ (Brandle et al., 1997) (Fig. 1A). To identify the cell type and subcellular distribution of purinergic receptors in olfactory epithelium and olfactory bulb, antibodies against $P2X_1$, $P2X_2$, $P2X_4$ and $P2Y_2$ receptors, and olfactory marker protein (OMP), which is found in mature olfactory receptor neurons (ORNs) were used. OMP-positive ORNs showed punctate immunoreactivity (IR) to $P2X_1$ and $P2X_4$ antibodies on cell somas and axons (Fig. 1B, C) and $P2Y_2$-IR on the dendrites, somas and axons (Fig. 1D). Both P2X- and P2Y-IR was absent from dendritic knobs and cilia of ORNs. OMP-negative ORNs and basal cells (olfactory stem cells) showed P2X- and P2Y-IR. Sustentacular cells and Bowman's glands showed only $P2Y_2$-IR (Fig 1D). In the olfactory bulb, there was $P2X_1$, $P2X_2$, $P2X_4$ and $P2Y_2$ receptor IR in the olfactory nerve layer, the glomerular layer and the mitral cell layer. There was no $P2X_2$-IR in the olfactory neuroepithelium; however, there was punctate $P2X_2$-IR on blood vessels just below the basal cells. Thus, the underlying blood vessels are the likely source of $P2X_2$ mRNA identified by the RT-PCR studies of the olfactory epithelium. Preabsorption of the primary antibody with peptide antigen (Fig. 1E) or omission of the primary antibody blocked the purinergic receptor staining. Identification of regionally localized purinergic receptors in mammalian olfactory epithelium is consistent with extracellular ATP playing multiple roles in the peripheral olfactory system.

(2) Purinergic Receptors are Functional In Cultured Olfactory Receptor Neurons

The physiological activation of purinergic receptors in cultured mouse ORNs (Vargas and Lucero, 1999a) were examined using both electrophysiology (Danaceau and Lucero, 1998) (Fig. 2A), and calcium imaging (Fig. 2B-E). ATP (10 μM) evoked inward currents in 39% (27/69 ORNs) of the perforated-patched mouse ORNs during brief (1-10 s) applications (Fig. 2A). Some cells exhibited a distinct, long latent period consistent with slowly activating G-protein coupled P2Y receptors (cell 1, Fig. 2A inset) (13/27 cells; latency = 1140 ± 236 ms; I_{max} = -29 ± 8 pA). Rapid activation of inward current that closely followed the ATP stimulus profile with little or no desensitization was also observed, indicating involvement of non-desensitizing ionotropic P2X receptors [$P2X_4$ and/or $P2X_7$] (Ralevic and Burnstock, 1998) (Fig. 2A, cell 2)

(14/27 cells; latency = 81 ± 15 ms, I_{max} = -235 ± 74 pA). These electrophysiological results support the immunohistochemical evidence of expression of both purinergic receptor subtypes in ORNs.

Extracellularly applied ATP evoked a rapid transient increase in $[Ca^{2+}]_i$ (Fig. 2B, C; 76/84 ORNs). On average, ATP (10 μM) induced a 151 ± 12% Δ fluorescence (F)/F increase in $[Ca^{2+}]_i$ (n = 76; range 13 - 398%). Averaged dose-response relations for ATP-induced $[Ca^{2+}]_i$ increases in cultured mouse ORNs gave half-maximally effective concentrations (EC_{50}) of 1.6 μM (n = 58), comparable to previous reports for brain P2X receptors (Fig. 2D) (North and Barnard, 1997;North and Surprenant, 2000;Ralevic and Burnstock, 1998). In the absence of external Ca^{2+}, the ATP-induced rise in $[Ca^{2+}]_i$ was 31 ± 11% larger than calcium transients in the presence of Ca^{2+}, although in a few ORNs (3/19), the ATP-evoked increases were smaller, but never absent, in Ca^{2+}-free solution (Fig. 2E). The increase in fluorescence intensity in the absence of Ca^{2+}indicates that (1) part of the signal results from release from intracellular Ca^{2+} stores, implicating P2Y receptor activation, and (2) Ca^{2+} can reduce the concentration of free ATP or modulate the purinergic receptor (Honore et al., 1989;North and Surprenant, 2000). Thus, electrophysiology and Ca^{2+} imaging show that purinergic receptors are functional in primary cultures of mouse ORNs.

(3) Confocal Imaging of Olfactory Epithelium Slices

To study the effects of ATP on odor responses, acutely prepared slices of mouse olfactory epithelium were used. Confocal imaging of fluo-4 AM-loaded olfactory epithelium slices allows simultaneous recording from identified structures within the olfactory epithelium, i.e., both ORNs and sustentacular cells. Reproducible odor-evoked calcium transients were obtained when imaging ORNs >100 μM below the surface of the slice (Fig. 3A1-A5). Odor-evoked calcium transients rapidly activated and returned to basal levels within 125.7 ± 11.1 s (Fig. 3A, n = 11 ORNs). Superfusion of ATP (10 μM) onto this slice evoked calcium transients from all 11 ORNs previously identified by their response to odors, although the responses to ATP (Fig. B1-B4) are less obvious than the odor responses (Fig. 3B2). The difference in robustness can be due to poor access to the ATP stimulus: odorant receptors are on the cilia of the ORNs, which extend beyond the outer edge of the olfactory epithelium, whereas purinergic receptors are located deeper within the olfactory epithelium. ATP also evoked calcium transients from sustentacular cells, identified by their location, morphology, and lack of response to odor (Fig. 3B3). The latency of activation for the ATP-evoked calcium transient was shorter in the ORN (Fig. 3B2, solid up arrowhead) than in sustentacular cells (Fig. 3B3, open down arrowhead). Collectively, this was consistent with ORNs expressing the faster P2X receptors, and the sustentacular cells expressing the slower G-protein coupled P2Y receptors.

To further test whether functional purinergic receptor subtypes are differentially expressed in olfactory epithelium cell types, purinergic receptor agonists were used. As there are no completely specific purinergic receptor agonists (Ralevic and Burnstock, 1998), the selectivity was determined as discussed herein. The P2X 'selective' agonist βγ-methylene ATP was superfused onto the slice (Fig. 3C). Only the ORNs, and not the sustentacular cells, responded to βγ-methylene ATP with an increase in $[Ca^{2+}]_i$ (Fig. 3C5). The P2Y 'selective' agonist UTP evoked calcium transients in both ORNs and sustentacular cells (Fig. 3D). However, compared to the non-selective agonist ATP, the peak amplitudes were smaller in the ORNs and the latency of activation in the ORNs was longer and equivalent to the latency of activation in the sustentacular cells

(compare Fig. 3D2,5 to B2,5). These data indicated that the ORN expressed P2X and, to a lesser extent, P2Y receptors, and that the sustentacular cells expressed only P2Y receptors. It further confirms the immunohistochemical and electrophysiological evidence for differential expression of purinergic receptors in mammalian olfactory epithelium.

A variety of non-selective purinergic receptor agonists (ATP, ATPγS, AMP), P2Y-'selective' agonists (UTP, ADP, MeS-ADP), P2X-'selective' agonists (βγ-methylene ATP) (Fig. 4A, B), and an adenosine receptor 'selective' agonist (adenosine) were superfused onto olfactory epithelial slices and the change in $[Ca^{2+}]_i$ was measured. Adenosine- or AMP-evoked calcium transients were never observed. ORNs responded with approximately equal frequency to P2Y and P2X receptor agonists whereas sustentacular cells responded primarily to P2Y receptor agonists. The general non-specific purinergic receptor antagonists Suramin and pyridoxalphosphate-6-azophenyl-2',4'-disulfonic acid (PPADS) were used to further confirm that purinergic-evoked calcium transients were mediated via purinergic receptors. In ORNs previously shown to respond to both ATP and UTP, Suramin (100 μM) reversibly blocked both ATP- and UTP-evoked calcium transients by 88 ± 2 and 72 ± 6% (n = 9). PPADS (25 μM) also reversibly blocked the ATP- and UTP-evoked calcium transients by 87 ± 5 and 92 ± 3% (n = 5). Purinergic receptor antagonists also reversibly blocked purinergic nucleotide-evoked calcium transients in sustentacular cells. Suramin blocked ATP- and UTP-evoked Ca^{2+} transients by 90 ± 1 and 89 ± 1% (n = 37) and PPADS blocked the transients by 82 ± 2 and 76 ± 2%, respectively (n = 30). Collectively, the data show that the ATP-evoked calcium transients were mediated by P2X and P2Y receptors.

(4) ATP Modulates Odor Responses

Calcium is an important intracellular messenger during odor transduction affecting signal amplification (Lowe and Gold, 1993) and adaptation (Zufall et al., 1991). The data indicate that purinergic nucleotides evoke robust increases in intracellular calcium. Odors were sequentially superfused, ATP and the combination of odors and ATP onto olfactory epithelium slice preparation. It was found that ATP could (1) have no effect, (2) cause suppression (Fig. 5A), where the calcium transient evoked by co-application is less than the sum of the calcium transients evoked by the individual components, or (3) cause enhancement (Fig. 5B), in which the calcium transient due to co-application is larger than the sum of the individual components. The increases in Ca^{2+} elicited from co-application of ATP and odor from two cells (Fig. 5A, B) have been expressed as a proportion of the sum of the individual responses (Fig. 5C). Of the cells that responded to odor, 62% exhibited a significant decrease in the summed $[Ca^{2+}]_i$ increase (mean suppression = 57 ± 5%; paired t-test, p = 0.01, n = 26). The observed decrease due to co-application was not the result of run-down because post-co-application responses both to ATP and to odors were ± 10% of pre-co-application (Fig. 5A). Of the odor-responsive ORNs, only two exhibited a >20% increase in evoked $[Ca^{2+}]_i$ increase (mean enhancement = 157 ± 34%), indicating a combined effect. Thus, ATP significantly reduced odor-induced calcium responses in the majority of ORNs.

(5) Activation Of Purinergic Receptor Subtypes Modulates Odor Sensitivity

The observation of both suppressive and combined responses indicates that ATP can modulate odor responses via activation of different purinergic receptor subtypes. This hypothesis was tested by sequentially superfusing odors, various selective purinergic receptor agonists and the combination of odors and purinergic receptor agonists onto the olfactory epithelium slice preparation. We found that co-application of the P2X agonist βγ-methylene ATP (10 μM) and odor (1) enhanced the calcium transient amplitude by 168 ± 44% (2/16 cells; Figures 6A-B), (2) had no effect on amplitude (15 ± 1%; 2/16 cells; data not shown), or (3) suppressed the amplitude of Ca^{2+} transient by 42 ± 5% (12/16 cells; Figures 6C-D). Overall, there is a statistically significant 25 ± 11% reduction in the average amplitude of the Ca^{2+} transient by co-application of odors and βγ-methylene ATP (16/16 cells; $p<0.04$, paired Student's t-test), even when including the 2 cells that did not show a significant change and the 2 cells that had an enhanced response. In contrast, co-application of the P2Y agonist ADPβS (10 μM) and odor suppressed the Ca^{2+} transient amplitude by 41 ± 4% (15/15 cells; $p<0.001$, paired Student's t-test; Fig 6E-F). Thus, the P2Y agonist ADPβS reduced the odor responsiveness of ORNs in all cells tested. In contrast, βγ-methylene ATP, like ATP, enhanced the odor responsiveness due to the activation of P2X receptors in a few cells. However, in the majority of ORNs, the P2X specific agonist significantly reduced odor-induced Ca^{2+} transients.

Disclosed herein, purinergic receptor subtypes are differentially expressed in ORNs and sustentacular cells, and ORNs express multiple purinergic receptor subtypes. In other cell types, expression of more than one type of purinergic receptor allows for regulation of multiple effectors and modification of agonist-evoked responses, and provides a mechanism for rapid and local fine tuning at the cellular level (Ralevic and Burnstock, 1998). Disclosed immunohistochemical studies showed a notable absence of purinergic receptors in the dendritic knobs and cilia, the site of odor transduction, whereas both P2X and P2Y receptors are located on cell somas and other regions. This indicates that purinergic receptor activation is unlikely to affect initial odor-induced receptor potentials, but can shape the final integrated output of the cell. Extracellular purine nucleotides have been reported to exert multiple trophic actions in the central nervous system (Neary et al., 1996). Because the olfactory neuroepithelium is constantly exposed to airborne pollutants and microbes, it continuously regenerates; different populations of neurons are in various stages of development, including birth, maturation, and programmed cell death or apoptosis (Graziadei and Monti-Graziadei, 1978). Thus, ATP released by acutely injured cells could act as an early signal of cell and tissue damage, and, due to the mitogenic and growth-promoting effects of purinergic receptor activation, stimulate regeneration. Growth promotion can be mediated by P2Y receptors, which, like other growth factor receptors, induce a cascade of intracellular events that trigger cell proliferation (Neary et al., 1996).

A longstanding dogma, that odor sensitivity is not modulated at the level of the olfactory epithelium, is based on anatomical studies showing absence of efferent synapses on ORNs(Getchell, 1986;Graziadei, 1971). A recent study showing release of ATP in the olfactory epithelium following noxious stimuli (Kilgour et al., 2000) provides a physiological source for a neuromodulatory substance that does not require efferent innervation.

b) Experimental Procedures

(1) RT-PCR

Total RNA was isolated from rat olfactory epithelium using Trizol (GIBCO BRL). Polyadenylated mRNA was selected using a cellulose oligo(dT) matrix (QuickPrep® Micro mRNA purification kit, Amersham Pharmacia Biotech). First-strand cDNA was prepared from 40 ng mRNA using SuperScript™ II RNase H- RT according to GIBCO BRL procedures. A control reaction omitting the reverse transcriptase was included to confirm absence of genomic contamination. First strand cDNA was amplified using Platinum®Taq DNA polymerase (Gibco BRL). Primers for detection of $P2X_2$ transcripts were 756-775 sense and 1537-1558 antisense oligonucleotides (accession #U14414)(Brake et al., 1994), primers for $P2Y_2$ transcripts were 1288-1307 sense and 1931-1950 antisense oligonucleotides (accession #U09402), primers for β-actin transcripts (Lopez-Candales et al., 1995) were 1038-1067 sense and 1875-1905 antisense oligonucleotides and primers for neuron specific enolase were 348-368 sense and 1101-1123 antisense oligonucleotides (accession #M11931). All the primer pairs (100 μM) were used with a 30-cycle profile performed as follows: 94°C denaturation (2 min), 96°C denaturation (45 s), 60°C annealing (1 min) and 72°C extension (1.5 min). PCR products were separated and visualized using ethidium bromide-stained agarose gels (1%). A semi-nested PCR protocol was used for detection of the $P2X_2$ receptor transcript. PCR products were excised from the gel and reamplified for 28 cycles using the same antisense primer and a sense primer corresponding to position 1059-1078. PCR products were sequenced at the University of Utah Sequencing Center.

(2) Immunohistochemistry.

All animal procedures were approved by the University of Utah Institutional Animal Care and Use Committee, and all applicable guidelines from the National Institutes of Health Guide for Care and Use of Laboratory Animals were followed. Olfactory epithelium from post-natal day 4 mice was dissected and post-fixed for 2 hours and then cryoprotected, oriented in Tissue Tek OCT and quickly frozen. Cryostat sections (14 μm) were permeabilized with 0.3% triton X-100 in PBS, blocked with 10% normal donkey serum. Double-labeling was performed by simultaneously incubating slices in goat anti-OMP (1:10K; generous gift from F. Margolis) and either rabbit anti- $P2X_1$, $P2X_2$, $P2X_4$, $P2Y_2$ (all 1:100; Alomone Labs, Jerusalem, Israel), or $P2X_2$ (3 mg/ml; Oncogene Research Products, Boston, MA) overnight followed by a 30 min. incubation in TRITC-conjugated donkey anti-goat immunoglobin secondary antibody plus FITC-conjugated donkey anti-rabbit immunoglobin secondary antibody (1:100) (both from Jackson ImmunoResearch Labs, West Grove, PA). For pre-absorption controls, P2 antibodies were incubated with a saturating amount of peptide immunogen (10X) for 1-2 hours and visualized as above.

(3) Olfactory Epithelium Slices and Primary Cultures.

To prepare olfactory epithelium slices, neonatal mice (postnatal day 0-6) were quickly decapitated, and the skin and lower jaw were removed. Tissue was mounted in ice cold Ringers onto a vibratome-cutting block and 300 μm slices were made. Primary cultures of mouse ORNs were made using the same protocol and culture conditions as described for rat olfactory receptor neurons (Vargas and Lucero, 1999a). Briefly, tissue was placed in divalent cation-free Ringers containing 10 mg/ml bovine serum albumin, 1 mg/ml deoxyribonuclease II and 44 U/ml dispase, incubated at 37°C for 45 min. The tissue was washed, triturated,

and filtered through a 53 mm mesh and 200 ml cells were plated onto concanavalin A-coated coverslips and allowed to settle for 20 min. An additional 1.5 ml of culture medium was added (DMEM supplemented with 100 mM ascorbic acid, 1X insulin-transferrin-seleniumX (GIBCO BRL), 2 mM glutamine, 100 U/ml penicillin G and 100 mg/ml streptomycin).

(4) Electrophysiology.

The nystatin perforated-patch technique (Horn and Marty, 1988) was used to examine cells under voltage-clamp. Electrodes (2 –5 MΩ) were filled with TMA-oxide internal solution (in mM: 62.5 TMA oxide, 62.5 KH_2PO_4, 15 KCl, 5 $MgCl_2$, 11 EGTA, 10 HEPES, 1 glutathione, 5 TEA, 0.03% pluronic acid F-127, 0.3% DMSO, 150 mg/ml nystatin, pH 7.2, 330 mOsm).

Electro-olfactogram and on-cell recordings: Slices of P0–P6 mouse OE were prepared as described above and mounted in a perfusion chamber with a bath flow of 3 ml/min. Test chemicals were introduced using a rotary injection valve (Rheodyne, Cotati, CA). The electro-olfactogram (EOG) recording electrode (3 M NaCl in1%agar; tip diameter, 5–10 um) was positioned along the dorsal portion of the nasal septum. The differential electrode (identical to the recording electrode) was positioned over skull cartilage and an $Ag^2/AgCl_2$ ground electrode was connected to the bath solution via a 3 M KCl agar bridge. Responses to test agents were amplified (5000 X gain) and filtered (2 kHz) by a low-noise differential DC amplifier. Data was digitized (100 Hz) using Axoscope 8.0 software (Axon Instruments).

For the noninvasive on-cell recordings (Chiu et al., 1997), the same electronics were used as described for nystatin-patch experiments. The recording electrode (5–8 M_ resistance) contained Ringer's solution. Test solutions were selected using a rotary valve and delivered for 30 sec using gravity flow. The time course of solution delivery was determined by placing an electrode in a slice and switching from Ringer's solution to distilled water. There was a 3 sec delay to initial electrical response, which peaked at 10 sec. The shaded region in Figure 8 shows the 30 sec window of when the valve was switched on and off. During a recording, the electrode was lowered into the dorsal septal region of the slice and a seal (0.5–1 Gohms) was made in voltage-clamp before switching to currentclamp with zero applied current. Only cells with a stable baseline were used. There was a 7 min wash between each test application. Experiments were conducted on 65 cells in 42 slices obtained from 14 P0–P6 mice from three litters. Only three cells survived long enough to complete the recovery portion of the >27 min protocol.

(5) Confocal Calcium Imaging.

$[Ca^{2+}]_i$ was determined using confocal imaging of fluo-4 AM (18 μM; Molecular Probes) loaded cells and slices. Cells or slices were placed in a laminar flow chamber (Warner Instruments) and perfused continuously with Ringers solution at a flow rate of 1.5-2.0 ml/min. Ringers contained (mM): 140 NaCl, 5 KCl, 1 $MgCl_2$, 2 $CaCl_2$, 10 HEPES, 10 glucose, 500 probenicid and 400 nM tetrodotoxin. Test solutions were applied using bath exchange and a small volume loop injector (200 μl). A Zeiss LSM 510 confocal laser scanning system was used for data collection and analysis. Time series experiments were performed collecting 1400 x 700 pixel images at 0.2-0.4 Hz. The fluorometric signals obtained are expressed as relative

fluorescence change, $\Delta F/F = (F-F_0)/F_0$, where F_0 is the basal fluorescence level. Increases in F greater than 10% above baseline noise were considered responses.

2. Example 2 Purinergic Receptor Antagonists Potentiate Odor Sensitivity

If the predominate role of endogenous ATP is to reduce odor sensitivity then addition of purinergic receptor antagonists should potentiate odor responses. Control experiments in which we applied odors at 5 - 8 min intervals revealed a small linear increase in the peak amplitude of the odor-induced Ca^{2+} transient (Fig. 12A & C). A linear regression between the first and last odor application was performed and the predicted amplitude of the middle response was calculated. Based on linear regressions, the actual amplitude of the middle odor application was not significantly different from the predicted amplitude, both in the single cell shown in Fig. 12A and in the average of 30 cells in Fig. 12C ($n = 30$; $-3 \pm 4\%$ difference; paired Student's t-test, $p = 0.47$). In contrast, when the middle odor application was preceded by and concomitant with perfusion of purinergic receptor antagonists (100 μM suramin + 25 μM PPADS), a significant increase in the Ca^{2+} transient amplitude such that the mean observed response was 14 ± 5 % larger than the predicted ($n = 22$; paired Student's t-test, $p = 0.024$). The differences between predicted and observed were statistically different when the control group was compared to the purinergic receptor agonist-treated group (independent Students t-test, $p = 0.012$). Application of purinergic receptor antagonists alone did not evoke calcium transients (Fig. 12B second trace). The elevated odor-evoked calcium transients would be expected if basal extracellular ATP were habitually reducing ORN sensitivity. Thus, the data shows that both endogenous and exogenous ATP reduces the amplitude of odor-evoked calcium transients through purinergic receptors, suggesting that ATP modulates ORN sensitivity.

3. Example 3 ATP Reduces Cyclic Nucleotide-Induced Electrical Responses

Odor activation of G-protein-coupled receptors results in increased cAMP production, opening of cyclic nucleotide-gated channels, influx of Ca^{2+} and Na^+, depolarization of the membrane, and activation of voltage- and Ca^{2+}-gated ion channels (Schild and Restrepo, 1998). Based on calcium imaging data, purinergics can reduce the odor-evoked electrical activity of ORNs. Recording odor-evoked membrane responses from single ORNs has a low probability of success because each ORN expresses only one or a few odorant receptors (Buck and Axel, 1991). Thus, a mixture of cyclic nucleotide modulators were used to record membrane responses: IBMX (100 μM), a phosphodiesterase inhibitor that prevents the breakdown of cAMP, CPT-cAMP (50 μM), and 8-Br-cGMP (50 μM), both membrane-permeant analogs of cAMP and cGMP, respectively. This cyclic nucleotide mixture was tested initially to verify that it evoked membrane potential changes in the OE slice preparation. The EOG measures field potential changes across the OE after stimulation. Similar EOG responses were obtained from both odor (10 μM) and the cyclic nucleotide mixture (Fig. 13A), validating the replacement of odor with the mixture in subsequent recordings. Next on-cell current-clamp recordings of ORNs were performed from neonatal mouse slices. The cyclic nucleotide mixture, the mixture + ATP, a second application of the mixture, and ATP was sequentially superfused onto a slice preparation and membrane potential changes were measured (Fig. 13B). The coapplication of ATP and the cyclic nucleotide mixture suppressed the cyclic nucleotide-induced electrical responses. The membrane response from each ORN was integrated from baseline and normalized to the initial cyclic nucleotide mixture

response. The presence of ATP reduced the electrical activity of the ORN by 67±2% (Fig. 13C)(n = 3 cells; p < 0.01, Newman-Keuls *post hoc* test). These data show that ATP modulates odor sensitivity in mammalian olfactory neurons.

G. Sequences

1. SEQ ID NO:1 The following is the sequence for H.sapiens mRNA for ATP receptor P2X1 (accession number X83688). Other sequences have been published for P2X1 receptors from rat vas deferens (accession number X80477) and mouse urinary bladder (accession number X84896).

```
   1 gaattcggct gatcccgcgg caggtgctag caggagctgg cagcatgggc tccccagggg
  61 ctacgacagg ctgggggctt ctggattata agacggagaa gtatgtgatg accaggaact
 121 ggcgggtggg cgccctgcag aggctgctgc agtttgggat cgtggtctat gtggtagggt
 181 gggctctcct cgccaaaaaa ggctaccagg agcgggacct ggaaccccag ttttccatca
 241 tcaccaaact caaaggggtt tccgtcactc agatcaagga gcttggaaac cggctgtggg
 301 atgtggccga cttcgtgaag ccacctcagg gagagaacgt gttcttcttg gtgaccaact
 361 tccttgtgac gccagcccaa gttcagggca gatgcccaga gcacccgtcc gtcccactgg
 421 ctaactgctg ggtcgacgaa gactgccccg aaggggaggg aggcacacac agccacggtg
 481 taaaaacagg ccagtgtgtg gtgttcaatg ggacccacag gacctgtgag atctggagtt
 541 ggtgcccagt ggagagtggc gttgtgccct cgaggccccc gctggcccag gcccagaact
 601 tcacactgtt catcaaaaac acagtcacct tcagcaagtt caacttctct aagtccaatg
 661 ccttggagac ctgggacccc acctatttta agcactgccg ctatgaacca caattcagcc
 721 cctactgtcc cgtgttccgc attggggacc tcgtggccaa ggctggaggg accttcgagg
 781 acctggcgtt gctgggtggc tctgtaggca tcagagttca ctgggattgt gacctggaca
 841 ccggggactc tggctgctgg cctcactact ccttccagct gcaggagaag agctacaact
 901 tcaggacagc cactcactgg tgggagcaac cgggtgtgga ggcccgcacc ctgctcaagc
 961 tctatggaat ccgcttcgac atcctcgtca ccgggcaggc agggaagttc gggctcatcc
1021 ccacggccgt cacactgggc accggggcag cttggctggg cgtggtcacc tttttctgtg
1081 acctgctact gctgtatgtg gatagagaag cccatttcta ctggaggaca aagtatgagg
1141 aggccaaggc cccgaaagca accgccaact ctgtgtggag ggagctggcc tttgcatccc
1201 aagcccgact ggccgagtgc ctcagacgga gctcagcacc tgcacccacg gccactgctg
1261 ctgggagtca gacacagaca ccaggatggc cctgtccaag ttctgacacc cacttgccaa
1321 cccattccgg gagcctgtag ccgtttccct gctggttgag aagagagagg ggctgggcaa
1381 ggaaggaccc ctgccctgcc gagcgaaagc aaggatgagg caacagcaat gaaagaagat
1441 caagccgaat tc
```

2. SEQ ID NO:2 The following is the sequence for H.sapiens protein for ATP receptor P2X1 (accession number X83688).

```
MARRFQEELAAFLFEYDTPRMVLVRNKKVGVIFRLIQLVVLVYV
IGWVFLYEKGYQTSSGLISSVSVKLKGLAVTQLPGLGPQVWDVADYVFPAQGDNSFVV
MTNFIVTPKQTQGYCAEHPEGGICKEDSGCTPGKAKRKAQGIRTGKCVAFNDTVKTCE
IFGWCPVEVDDDIPRPALLREAENFTLFIKNSISFPRFKVNRRNLVEEVNAAHMKTCL
FHKTLHPLCPVFQLGYVVQESGQNFSTLAEKGGVVGITIDWHCDLDWHVRHCRPIYEF
HGLYEEKNLSPGFNFRFARHFVENGTNYRHLFKVFGIRFDILVDGKAGKFDIIPTMTT
IGSGIGIFGVATVLCDLLLLHILPKRHYYKQKKFKYAEDMGPGAAERDLAATSSTLGL
QENMRTS
```

3. SEQ ID NO:3 The following sequence for the P2X2 receptor is derived from rat PC12 cells (accession number U14414). Other sequences have been published for P2X2 receptors from rat cerebellum (accession number Y09910)

```
   1 gaattcggct gatcccgcgg caggtgctag caggagctgg cagcatgggc tccccagggg
  61 ctacgacagg ctgggggctt ctggattata agacggagaa gtatgtgatg accaggaact
 121 ggcgggtggg cgccctgcag aggctgctgc agtttgggat cgtggtctat gtggtagggt
 181 gggctctcct cgccaaaaaa ggctaccagg agcgggacct ggaaccccag ttttccatca
 241 tcaccaaact caaaggggtt tccgtcactc agatcaagga gcttggaaac cggctgtggg
 301 atgtggccga cttcgtgaag ccacctcagg gagagaacgt gttcttcttg gtgaccaact
 361 tccttgtgac gccagcccaa gttcagggca gatgcccaga gcacccgtcc gtcccactgg
```

```
 421 ctaactgctg ggtcgacgaa gactgccccg aaggggaggg aggcacacac agccacggtg
 481 taaaaacagg ccagtgtgtg gtgttcaatg ggacccacag gacctgtgag atctggagtt
 541 ggtgcccagt ggagagtggc gttgtgccct cgaggcccct gctggcccag gcccagaact
 601 tcacactgtt catcaaaaac acagtcacct tcagcaagtt caacttctct aagtccaatg
 661 ccttggagac ctgggacccc acctatttta agcactgccg ctatgaacca caattcagcc
 721 cctactgtcc cgtgttccgc attggggacc tcgtggccaa ggctggaggg accttcgagg
 781 acctggcgtt gctgggtggc tctgtaggca tcagagttca ctgggattgt gacctggaca
 841 ccggggactc tggctgctgg cctcactact ccttccagct gcaggagaag agctacaact
 901 tcaggacagc cactcactgg tgggagcaac cgggtgtgga ggcccgcacc ctgctcaagc
 961 tctatggaat ccgcttcgac atcctcgtca ccgggcaggc agggaagttc gggctcatcc
1021 ccacggccgt cacactgggc accggggcag cttggctggg cgtggtcacc tttttctgtg
1081 acctgctact gctgtatgtg gatagagaag cccatttcta ctggaggaca aagtatgagg
1141 aggccaaggc cccgaaagca accgccaact ctgtgtggag ggagctggcc tttgcatccc
1201 aagcccgact ggccgagtgc ctcagacgga gctcagcacc tgcacccacg gccactgctg
1261 ctgggagtca gacacagaca ccaggatggc cctgtccaag ttctgacacc cacttgccaa
1321 cccattccgg gagcctgtag ccgtttccct gctggttgag aagagagagg ggctgggcaa
1381 ggaaggaccc ctgccctgcc gagcgaaagc aaggatgagg caacagcaat gaaagaagat
1441 caagccgaat tc
```

4. **SEQ ID NO:4 The following sequence for the P2X2 receptor is derived from rat PC12 cells (accession number U14414) protein sequence.**

```
MVRRLARGCWSAFWDYETPKVIVVRNRRLGFVHRMVQLLILLYF
VWYVFIVQKSYQDSETGPESSIITKVKGITMSEDKVWDVEEYVKPPEGGSVVSIITRI
EVTPSQTLGTCPESMRVHSSTCHSDDDCIAGQLDMQGNGIRTGHCVPYYHGDSKTCEV
SAWCPVEDGTSDNHFLGKMAPNFTILIKNSIHYPKFKFSKGNIASQKSDYLKHCTFDQ
DSDPYCPIFRLGFIVEKAGENFTELAHKGGVIGVIINWNCDLDLSESECNPKYSFRRL
DPKYDPASSGYNFRFAKYYKINGTTTTRTLIKAYGIRIDVIVHGQAGKFSLIPTIINL
ATALTSIGVGSFLCDWILLTFMNKNKLYSHKKFDKVRTPKHPSSRWPVTLALVLGQIP
PPPSHYSQDQPPSPPSGEGPTLGEGAELPLAVQSPRPCSISALTEQVVDTLGQHMGQR
PPVPEPSQQDSTSTDPKGLAQL
```

5. **SEQ ID NO:5 The following sequence for the P2X3 receptor is derived from H.sapiens (accession number Y07683). Other sequences have been published for P2X3 receptors from rat dorsal root ganglion (accession number X91167 and X90651).**

```
   1 gaattcggct gatcccgcgg caggtgctag caggagctgg cagcatgggc tccccagggg
  61 ctacgacagg ctgggggctt ctggattata agacggagaa gtatgtgatg accaggaact
 121 ggcgggtggg cgccctgcag aggctgctgc agtttgggat cgtggtctat gtggtagggt
 181 gggctctcct cgccaaaaaa ggctaccagg agcgggacct ggaaccccag ttttccatca
 241 tcaccaaact caaaggggtt tccgtcactc agatcaagga gcttggaaac cggctgtggg
 301 atgtggccga cttcgtgaag ccacctcagg gagagaacgt gttcttcttg gtgaccaact
 361 tccttgtgac gccagcccaa gttcagggca gatgcccaga gcacccgtcc gtcccactgg
 421 ctaactgctg ggtcgacgaa gactgccccg aaggggaggg aggcacacac agccacggtg
 481 taaaaacagg ccagtgtgtg gtgttcaatg ggacccacag gacctgtgag atctggagtt
 541 ggtgcccagt ggagagtggc gttgtgccct cgaggcccct gctggcccag gcccagaact
 601 tcacactgtt catcaaaaac acagtcacct tcagcaagtt caacttctct aagtccaatg
 661 ccttggagac ctgggacccc acctatttta agcactgccg ctatgaacca caattcagcc
 721 cctactgtcc cgtgttccgc attggggacc tcgtggccaa ggctggaggg accttcgagg
 781 acctggcgtt gctgggtggc tctgtaggca tcagagttca ctgggattgt gacctggaca
 841 ccggggactc tggctgctgg cctcactact ccttccagct gcaggagaag agctacaact
 901 tcaggacagc cactcactgg tgggagcaac cgggtgtgga ggcccgcacc ctgctcaagc
 961 tctatggaat ccgcttcgac atcctcgtca ccgggcaggc agggaagttc gggctcatcc
1021 ccacggccgt cacactgggc accggggcag cttggctggg cgtggtcacc tttttctgtg
1081 acctgctact gctgtatgtg gatagagaag cccatttcta ctggaggaca aagtatgagg
1141 aggccaaggc cccgaaagca accgccaact ctgtgtggag ggagctggcc tttgcatccc
1201 aagcccgact ggccgagtgc ctcagacgga gctcagcacc tgcacccacg gccactgctg
1261 ctgggagtca gacacagaca ccaggatggc cctgtccaag ttctgacacc cacttgccaa
1321 cccattccgg gagcctgtag ccgtttccct gctggttgag aagagagagg ggctgggcaa
1381 ggaaggaccc ctgccctgcc gagcgaaagc aaggatgagg caacagcaat gaaagaagat
1441 caagccgaat tc
```

6. SEQ ID NO:6 The following sequence for the P2X3 receptor is derived from H.sapiens (accession number Y07683) protein sequence.

MNCISDFFTYETTKSVVVKSWTIGIINRVVQLLIISYFVGWVFL
HEKAYQVRDTAIESSVVTKVKGSGLYANRVMDVSDYVTPPQGTSVFVIITKMIVTENQ
MQGFCPESEEKYRCVSDSQCGPEPLPGGGILTGRCVNYSSVLRTCEIQGWCPTEVDTV
ETPIMMEAENFTIFIKNSIRFPLFNFEKGNLLPNLTARDMKTCRFHPDKDPFCPILRV
GDVVKFAGQDFAKLARTGGVLGIKIGWVCDLDKAWDQCIPKYSFTRLDSVSEKSSVSP
GYNFRFAKYYKMENGSEYRTLLKAFGIRFDVLVYGNAGKFNIIPTIISSVAAFTSVGV
GTVLCDIILLNFLKGADQYKAKKFEEVNETTLKIAALTNPVYPSDQTTAEKQSTDSGA
FSIGH

7. SEQ ID NO:7 The following sequence for the P2X4 receptor is derived from H.sapiens (accession number Y07684). Other sequences have been published for P2X4 receptors from rat brain (accession number X93565, U32497, X91200 and X87763) and rat pancreatic islet (accession number U47031).

```
   1 gaattcggct gatcccgcgg caggtgctag caggagctgg cagcatgggc tccccagggg
  61 ctacgacagg ctgggggctt ctggattata agacggagaa gtatgtgatg accaggaact
 121 ggcgggtggg cgccctgcag aggctgctgc agtttgggat cgtggtctat gtggtagggt
 181 gggctctcct cgccaaaaaa ggctaccagg agcgggacct ggaaccccag ttttccatca
 241 tcaccaaact caaaggggtt tccgtcactc agatcaagga gcttggaaac cggctgtggg
 301 atgtggccga cttcgtgaag ccacctcagg gagagaacgt gttcttcttg gtgaccaact
 361 tccttgtgac gccagcccaa gttcagggca gatgcccaga gcacccgtcc gtcccactgg
 421 ctaactgctg ggtcgacgaa gactgccccg aaggggaggg aggcacacac agccacggtg
 481 taaaaacagg ccagtgtgtg gtgttcaatg ggacccacag gacctgtgag atctggagtt
 541 ggtgcccagt ggagagtggc gttgtgccct cgaggcccct gctggcccag gcccagaact
 601 tcacactgtt catcaaaaac acagtcacct tcagcaagtt caacttctct aagtccaatg
 661 ccttggagac ctgggacccc acctatttta agcactgccg ctatgaacca caattcagcc
 721 cctactgtcc cgtgttccgc attggggacc tcgtggccaa ggctggaggg accttcgagg
 781 acctggcgtt gctgggtggc tctgtaggca tcagagttca ctgggattgt gacctggaca
 841 ccggggactc tggctgctgg cctcactact ccttccagct gcaggagaag agctacaact
 901 tcaggacagc cactcactgg tgggagcaac cgggtgtgga ggcccgcacc ctgctcaagc
 961 tctatggaat ccgcttcgac atcctcgtca ccgggcaggc agggaagttc gggctcatcc
1021 ccacggccgt cacactgggc accggggcag cttggctggg cgtggtcacc tttttctgtg
1081 acctgctact gctgtatgtg gatagagaag cccatttcta ctggaggaca aagtatgagg
1141 aggccaaggc cccgaaagca accgccaact ctgtgtggag ggagctggcc tttgcatccc
1201 aagcccgact ggccgagtgc ctcagacgga gctcagcacc tgcacccacg gccactgctg
1261 ctgggagtca gacacagaca ccaggatggc cctgtccaag ttctgacacc cacttgccaa
1321 cccattccgg gagcctgtag ccgtttccct gctggttgag aagagagagg ggctgggcaa
1381 ggaaggaccc ctgccctgcc gagcgaaagc aaggatgagg caacagcaat gaaagaagat
1441 caagccgaat tc
```

8. SEQ ID NO:8 The following sequence for the P2X4 receptor is derived from H.sapiens (accession number Y07684) Protein sequence.

MAGCCSALAAFLFEYDTPRIVLIRSRKVGLMNRAVQLLILAYVI
GWVFVWEKGYQETDSVVSSVTTKVKGVAVTNTSKLGFRIWDVADYVIPAQEENSLFVM
TNVILTMNQTQGLCPEIPDATTVCKSDASCTAGSAGTHSNGVSTGRCVAFNGSVKTCE
VAAWCPVEDDTHVPQPAFLKAAENFTLLVKNNIWYPKFNFSKRNILPNITTTYLKSCI
YDAKTDPFCPIFRLGKIVENAGHSFQDMAVEGGIMGIQVNWDCNLDRAASLCLPRYSF
RRLDTRDVEHNVSPGYNFRFAKYYRDLAGNEQRTLIKAYGIRFDIIVFGKAGKFDIIP
TMINIGSGLALLGMATVLCDIIVLYCMKKRLYYREKKYKYVEDYEQGLASELDQ

9. SEQ ID NO:9 The following sequence for the P2X5 receptor is derived from H.sapiens (accession number AF016709). Other sequences have been published for P2X5 receptors from rat brain (accession number X92069) and rat heart (accession number X97328).

```
1 ggcacgaggg tccgcaagcc cggctgagag cgcgccatgg ggcaggcggg ctgcaagggg
```

```
  61 ctctgcctgt cgctgttcga ctacaagacc gagaagtatg tcatcgccaa gaacaagaag
 121 gtgggcctgc tgtaccggct gctgcaggcc tccatcctgg cgtacctggt cgtatgggtg
 181 ttcctgataa agaagggtta ccaagacgtc gacacctccc tgcagagtgc tgtcatcacc
 241 aaagtcaagg gcgtggcctt caccaacacc tcggatcttg ggcagcggat ctgggatgtc
 301 gccgactacg tcattccagc ccagggagag aacgtctttt ttgtggtcac caacctgatt
 361 gtgaccccca accagcggca gaacgtctgt gctgagaatg aaggcattcc tgatggcgcg
 421 tgctccaagg acagcgactg ccacgctggg gaagcggtta cagctggaaa cggagtgaag
 481 accggccgct gcctgcggag agggaacttg gccaggggca cctgtgagat ctttgcctgg
 541 tgcccgttgg agacaagctc caggccggag gagccattcc tgaaggaggc cgaagacttc
 601 accattttca taaagaacca catccgtttc cccaaattca acttctccaa aaacaatgtg
 661 atggacgtca aggacagatc tttcctgaaa tcatgccact ttggccccaa gaaccactac
 721 tgccccatct tccgactggg ctccatcgtc cgctgggccg ggagcgactt ccaggatata
 781 gccctgcgag gtggcgtgat aggaattaat attgaatgga actgtgatct tgataaagct
 841 gcctctgagt gccaccctca ctattctttt agccgtctgg acaataaact ttcaaagtct
 901 gtctcctccg ggtacaactt cagatttgcc agatattacc gagacgcagc cggggtggag
 961 ttccgcaccc tgatgaaagc ctacgggatc cgctttgacg tgatggtgaa cggcaagggt
1021 gctttcttct gcgacctggt actcatctac ctcatcaaaa agagagagtt ttaccgtgac
1081 aagaagtacg aggaagtgag gggcctagaa gacagttccc aggaggccga ggacgaggca
1141 tcgggggctgg ggctatctga gcagctcaca tctgggccag ggctgctggg gatgccggag
1201 cagcaggagc tgcaggagcc acccgaggcg aagcgtggaa gcagcagtca gaaggggaac
1261 ggatctgtgt gcccacagct cctggagccc cacaggagca cgtgaattgc ctctgcttac
1321 gttcaggccc tgtcctaaac ccagccgtct agcacccagt gatcccatgc ctttgggaat
1381 cccaggatgc tgcccaacgg gaaatttgta cattgggtgc tatcaatgcc acatcacagg
1441 gaccagccat cacagagcaa agtgacctcc acgtctgatg ctggggtcat caggacggac
1501 ccatcatggc tgtctttttg ccccaccccc tgccgtcagt tcttcctttc tccgtggctg
1561 gcttcccgca ctagggaacg ggttgtaaat ggggaacatg acttccttcc ggagtccttg
1621 agcacctcag ctaaggaccg cagtgccctg tagagttcct agattacctc actgggaata
1681 gcattgtgcg tgtccggaaa agggctccat ttggttccag cccactcccc tctgcaagtg
1741 ccacagcttc cctcagagca tactctccag tggatccaag tactctctct cctaaagaca
1801 ccaccttcct gccagctgtt tgcccttagg ccagtacaca gaattaaagt gggggagatg
1861 gcagacgctt tctgggacct gcccaagata tgtattctct gacactctta tttggtcata
1921 aaacaataaa tggtgtcaat ttcaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaa
```

10. SEQ ID NO:10 The following sequence for the P2X5 receptor is derived from H.sapiens (accession number AF016709) protein sequence.

```
MGQAGCKGLCLSLFDYKTEKYVIAKNKKVGLLYRLLQASILAYL
VVWVFLIKKGYQDVDTSLQSAVITKVKGVAFTNTSDLGQRIWDVADYVIPAQGENVFF
VVTNLIVTPNQRQNVCAENEGIPDGACSKDSDCHAGEAVTAGNGVKTGRCLRRGNLAR
GTCEIFAWCPLETSSRPEEPFLKEAEDFTIFIKNHIRFPKFNFSKNNVMDVKDRSFLK
SCHFGPKNHYCPIFRLGSIVRWAGSDFQDIALRGGVIGINIEWNCDLDKAASECHPHY
SFSRLDNKLSKSVSSGYNFRFARYYRDAAGVEFRTLMKAYGIRFDVMVNGKGAFFCDL
VLIYLIKKREFYRDKKYEEVRGLEDSSQEAEDEASGLGLSEQLTSGPGLLGMPEQQEL
QEPPEAKRGSSSQKGNGSVCPQLLEPHRST
```

11. SEQ ID NO:11. The following sequence for the P2X6 receptor is derived from H.sapiens (accession number AF065385). Other sequences have been published for P2X6 receptors from rat brain (accession numbers X92070 and X97376).

```
   1 gaattcggct gatcccgcgg caggtgctag caggagctgg cagcatgggc tccccagggg
  61 ctacgacagg ctgggggctt ctggattata agacggagaa gtatgtgatg accaggaact
 121 ggcgggtggg cgccctgcag aggctgctgc agtttgggat cgtggtctat gtggtagggt
 181 gggctctcct cgccaaaaaa ggctaccagg agcgggacct ggaaccccag ttttccatca
 241 tcaccaaact caaaggggtt tccgtcactc agatcaagga gcttggaaac cggctgtggg
 301 atgtggccga cttcgtgaag ccacctcagg gagagaacgt gttcttcttg gtgaccaact
 361 tccttgtgac gccagcccaa gttcagggca gatgcccaga gcacccgtcc gtcccactgg
 421 ctaactgctg ggtcgacgaa gactgccccg aaggggaggg aggcacacac agccacggtg
 481 taaaaacagg ccagtgtgtg gtgttcaatg ggacccacag gacctgtgag atctggagtt
 541 ggtgcccagt ggagagtggc gttgtgccct cgaggccccc gctggcccag gcccagaact
 601 tcacactgtt catcaaaaac acagtcacct tcagcaagtt caacttctct aagtccaatg
 661 ccttggagac ctgggacccc acctatttta agcactgccg ctatgaacca caattcagcc
 721 cctactgtcc cgtgttccgc attggggacc tcgtggccaa ggctggaggg accttcgagg
 781 acctggcgtt gctgggtggc tctgtaggca tcagagttca ctgggattgt gacctggaca
 841 ccgggggactc tggctgctgg cctcactact ccttccagct gcaggagaag agctacaact
 901 tcaggacagc cactcactgg tgggagcaac cgggtgtgga ggcccgcacc ctgctcaagc
```

```
 961 tctatggaat ccgcttcgac atcctcgtca ccgggcaggc agggaagttc gggctcatcc
1021 ccacggccgt cacactgggc accggggcag cttggctggg cgtggtcacc tttttctgtg
1081 acctgctact gctgtatgtg gatagagaag cccatttcta ctggaggaca aagtatgagg
1141 aggccaaggc cccgaaagca accgccaact ctgtgtggag ggagctggcc tttgcatccc
1201 aagcccgact ggccgagtgc ctcagacgga gctcagcacc tgcacccacg gccactgctg
1261 ctgggagtca gacacagaca ccaggatggc cctgtccaag ttctgacacc cacttgccaa
1321 cccattccgg gagcctgtag ccgtttccct gctggttgag aagagagagg ggctgggcaa
1381 ggaaggaccc ctgccctgcc gagcgaaagc aaggatgagg caacagcaat gaaagaagat
1441 caagccgaat tc
```

12. SEQ ID NO:12. The following sequence for the P2X6 receptor is derived from H.sapiens (accession number AF065385) protein sequence.

```
MGSPGATTGWGLLDYKTEKYVMTRNWRVGALQRLLQFGIVVYVV
GWALLAKKGYQERDLEPQFSIITKLKGVSVTQIKELGNRLWDVADFVKPPQGENVFFL
VTNFLVTPAQVQGRCPEHPSVPLANCWVDEDCPEGEGGTHSHGVKTGQCVVFNGTHRT
CEIWSWCPVESGVVPSRPLLAQAQNFTLFIKNTVTFSKFNFSKSNALETWDPTYFKHC
RYEPQFSPYCPVFRIGDLVAKAGGTFEDLALLGGSVGIRVHWDCDLDTGDSGCWPHYS
FQLQEKSYNFRTATHWWEQPGVEARTLLKLYGIRFDILVTGQAGKFGLIPTAVTLGTG
AAWLGVVTFFCDLLLLYVDREAHFYWRTKYEEAKAPKATANSVWRELAFASQARLAEC
LRRSSAPAPTATAAGSQTQTPGWPCPSSDTHLPTHSGSL
```

13. SEQ ID NO:13 The following sequence for the P2X7 receptor is derived from H.sapiens brain (accession number Y09561). Please note that other sequences have been published for P2X7 receptors from rat brain (accession numbers X95882)

```
   1 aaaacgcagg gagggaggct gtcaccatgc cggcctgctg cagctgcagt gatgttttcc
  61 agtatgagac gaacaaagtc actcggatcc agagcatgaa ttatggcacc attaagtggt
 121 tcttccacgt gatcatcttt tcctacgttt gctttgctct ggtgagtgac aagctgtacc
 181 agcggaaaga gcctgtcatc agttctgtgc acaccaaggt gaaggggata gcagaggtga
 241 aagaggagat cgtggagaat ggagtgaaga agttggtgca cagtgtcttt gacaccgcag
 301 actacacctt ccctttgcag gggaactctt tcttcgtgat gacaaacttt ctcaaaacag
 361 aaggccaaga gcagcggttg tgtcccgagt atcccacccg caggacgctc tgttcctctg
 421 accgaggttg taaaaaggga tggatggacc cgcagagcaa aggaattcag accggaaggt
 481 gtgtagtgca tgaagggaac cagaagacct gtgaagtctc tgcctggtgc cccatcgagg
 541 cagtggaaga ggccccccgg cctgctctct tgaacagtgc cgaaaacttc actgtgctca
 601 tcaagaacaa tatcgacttc cccggccaca actacaccac gagaaacatc ctgccaggtt
 661 taaacatcac ttgtaccttc cacaagactc agaatccaca gtgtcccatt ttccgactag
 721 gagacatctt ccgagaaaca ggcgataatt tttcagatgt ggcaattcag ggcggaataa
 781 tgggcattga gatctactgg gactgcaacc tagaccgttg gttccatcac tgccatccca
 841 aatacagttt ccgtcgcctt gacgacaaga ccaccaacgt gtccttgtac cctggctaca
 901 acttcagata cgccaagtac tacaaggaaa acaatgttga gaaacggact ctgataaaag
 961 tcttcgggat ccgttttgac atcctggttt ttggcaccgg aggaaaattt gacattatcc
1021 agctggttgt gtacatcggc tcaaccctct cctacttcgg tctggccgct gtgttcatcg
1081 acttcctcat cgacacttac tccagtaact gctgtcgctc ccatatttat ccctggtgca
1141 agtgctgtca gccctgtgtg gtcaacgaat actactacag gaagaagtgc gagtccattg
1201 tggagccaaa gccgacatta aagtatgtgt cctttgtgga tgaatcccac attaggatgg
1261 tgaaccagca gctactaggg agaagtctgc aagatgtcaa gggccaagaa gtcccaagac
1321 ctgcgatgga cttcacagat ttgtccaggc tgcccctggc cctccatgac acacccccga
1381 ttcctggaca accagaggag atacagctgc ttagaaagga ggcgactcct agatccaggg
1441 atagccccgt ctggtgccag tgtggaagct gcctcccatc tcaactccct gagagccaca
1501 ggtgcctgga ggagctgtgc tgccggaaaa agccgggggc ctgcatcacc acctcagagc
1561 tgttcaggaa gctggtcctg tccagacacg tcctgcagtt cctcctgctc taccaggagc
1621 ccttgctggc gctggatgtg gattccacca acagccggct gcggcactgt gcctacaggt
1681 gctacgccac ctggcgcttc ggctcccagg acatggctga ctttgccatc ctgcccagct
1741 gctgccgctg gaggatccgg aaagagtttc cgaagagtga agggcagtac agtggcttca
1801 agagtcctta ctgaagccag gcaccgtggc tcacgtctgt aatcccacct ttt
```

14. SEQ ID NO:14 The following sequence for the P2X7 receptor is derived from H.sapiens brain (accession number Y09561) protein sequence

```
MPACCSCSDVFQYETNKVTRIQSMNYGTIKWFFHVIIFSYVCFA
```

LVSDKLYQRKEPVISSVHTKVKGIAEVKEEIVENGVKKLVHSVFDTADYTFPLQGNSF
FVMTNFLKTEGQEQRLCPEYPTRRTLCSSDRGCKKGWMDPQSKGIQTGRCVVHEGNQK
TCEVSAWCPIEAVEEAPRPALLNSAENFTVLIKNNIDFPGHNYTTRNILPGLNITCTF
HKTQNPQCPIFRLGDIFRETGDNFSDVAIQGGIMGIEIYWDCNLDRWFHHCHPKYSFR
RLDDKTTNVSLYPGYNFRYAKYYKENNVEKRTLIKVFGIRFDILVFGTGGKFDIIQLV
VYIGSTLSYFGLAAVFIDFLIDTYSSNCCRSHIYPWCKCCQPCVVNEYYYRKKCESIV
EPKPTLKYVSFVDESHIRMVNQQLLGRSLQDVKGQEVPRPAMDFTDLSRLPLALHDTP
PIPGQPEEIQLLRKEATPRSRDSPVWCQCGSCLPSQLPESHRCLEELCCRKKPGACIT
TSELFRKLVLSRHVLQFLLLYQEPLLALDVDSTNSRLRHCAYRCYATWRFGSQDMADF
AILPSCCRWRIRKEFPKSEGQYSGFKSPY

15. SEQ ID NO:15 The following sequence for the P2Y1 receptor is derived from H.sapiens (accession number S81950). Other sequences have been published for P2Y1 receptors from human placenta (accession number Z49205), HEL cells (accession number U42030), bovine endothelium (accession numberX87628), rat cells (accession numbers U22830 and U22829), turkey brain (accession number U09842) and chicken brain (accession number X73268).

```
   1 ggatccagtt cgcctgctcc cttccgctcg ctggcttttc cgatgcttgc tgcgcccctg
  61 gccgccgctg ccctctcgcc gcctcctacc cctcggagcc gccgcctaag tcgaggagga
 121 gagaatgacc gaggtgctgt ggccggctgt ccccaacggg acggacgctg ccttcctggc
 181 cggtccgggt tcgtcctggg ggaacagcac ggtcgcctcc actgccgccg tctcctcgtc
 241 gttcaaatgc gccttgacca agacgggctt ccagttttac tacctgccgg ctgtctacat
 301 cttggtattc atcatcggct tcctgggcaa cagcgtggcc atctggatgt tcgtcttcca
 361 catgaagccc tggagcggca tctccgtgta catgttcaat ttggctctgg ccgacttctt
 421 gtacgtgctg actctgccag ccctgatctt ctactacttc aataaaacag actggatctt
 481 cggggatgcc atgtgtaaac tgcagaggtt catctttcat gtgaacctct atggcagcat
 541 cttgtttctg acatgcatca gtgcccaccg gtacagcggt gtggtgtacc ccctcaagtc
 601 cctgggccgg ctcaaaaaga agaatgcgat ctgtatcagc gtgctggtgt ggctcattgt
 661 ggtggtggcg atctccccca tcctcttcta ctcaggtacc ggggtccgca aaaacaaaac
 721 catcacctgt tacgacacca cctcagacga gtacctgcga agttatttca tctacagcat
 781 gtgcacgacc gtggccatgt tctgtgtccc cttggtgctg attctgggct gttacggatt
 841 aattgtgaga gctttgattt acaaagatct ggacaactct cctctgagga gaaaatcgat
 901 ttacctggta atcattgtac tgactgtttt tgctgtgtct tacatccctt tccatgtgat
 961 gaaaacgatg aacttgaggg cccggcttga ttttcagacc ccagcaatgt gtgctttcaa
1021 tgacagggtt tatgccacgt atcaggtgac aagaggtcta gcaagtctca acagttgtgt
1081 ggaccccatt ctctatttct tggcgggaga tactttcaga aggagactct cccgagccac
1141 aaggaaagct tctagaagaa gtgaggcaaa tttgcaatcc aagagtgaag acatgaccct
1201 caatatttta cctgagttca agcagaatgg agatacaagc ctgtgaaggc acaagaatct
1261 ccaaacacct ctctgttgta atatggtagg atgcttaaca gaatcaagta ct
```

16. SEQ ID NO:16 The following sequence for the P2Y1 receptor is derived from H.sapiens (accession number S81950).

MTEVLWPAVPNGTDAAFLAGPGSSWGNSTVASTAAVSSSFKCAL
TKTGFQFYYLPAVYILVFIIGFLGNSVAIWMFVFHMKPWSGISVYMFNLALADFLYVL
TLPALIFYYFNKTDWIFGDAMCKLQRFIFHVNLYGSILFLTCISAHRYSGVVYPLKSL
GRLKKKNAICISVLVWLIVVVAISPILFYSGTGVRKNKTITCYDTTSDEYLRSYFIYS
MCTTVAMFCVPLVLILGCYGLIVRALIYKDLDNSPLRRKSIYLVIIVLTVFAVSYIPF
HVMKTMNLRARLDFQTPAMCAFNDRVYATYQVTRGLASLNSCVDPILYFLAGDTFRRR
LSRATRKASRRSEANLQSKSEDMTLNILPEFKQNGDTSL

17. SEQ ID NO:17The following sequence for the P2Y2 receptor is derived from H.sapiens epithelial cells (accession number U07225). Other sequences have been published for P2Y2 receptors from rat alveolar cells (accession number U09402), rat pituitary cells (accession number L46865), Wistar Kyoto rat (accession number U56839), and mouse neuroblastoma cells (accession number NM_008773).

```
1 cggcacgagg caccccgaga ggagaagcgc agcgcagtgg cgagaggagc cccttgtggc
```

```
  61 agcagcacta cctgcccaga aaaatgctgg aggctgggcg tggccccagg cctggggacc
 121 tgtttttcct gtttcccgca gagttccctg cagcccggtc caggtccagg cgtgtgcatt
 181 catgagtgag gaacccgtgc aggcgctgag catcctgacc tggagagcag gggctggtca
 241 gggcgatggc agcagacctg ggcccctgga atgacaccat caatggcacc tgggatgggg
 301 atgagctggg ctacaggtgc cgcttcaacg aggacttcaa gtacgtgctg ctgcctgtgt
 361 cctacggcgt ggtgtgcgtg cttgggctgt gtctgaacgc cgtggcgctc tacatcttct
 421 tgtgccgcct caagacctgg aatgcgtcca ccacatatat gttccacctg gctgtgtctg
 481 atgcactgta tgcggcctcc ctgccgctgc tggtctatta ctacgcccgc ggcgaccact
 541 ggcccttcag cacggtgctc tgcaagctgg tgcgcttcct cttctacacc aacctttact
 601 gcagcatcct cttcctcacc tgcatcagcg tgcaccggtg tctgggcgtc ttacgacctc
 661 tgcgctccct gcgctggggc cgggcccgct acgctcgccg ggtggccggg gccgtgtggg
 721 tgttggtgct ggcctgccag gcccccgtgc tctactttgt caccaccagc gcgcgcgggg
 781 gccgcgtaac ctgccacgac acctcggcac ccgagctctt cagccgcttc gtggcctaca
 841 gctcagtcat gctgggcctg ctcttcgcgg tgccctttgc cgtcatcctt gtctgttacg
 901 tgctcatggc tcggcgactg ctaaagccag cctacgggac ctcgggcggc ctccctaggg
 961 ccaagcgcaa gtccgtgcgc accatcgccg tggtgctggc tgtcttcgcc ctctgcttcc
1021 tgccattcca cgtcacccgc accctctact actccttccg ctcgctggac ctcagctgcc
1081 acaccctcaa cgccatcaac atggcctaca aggttacccg gccgctggcc agtgctaaca
1141 gttgccttga ccccgtgctc tacttcctgg ctgggcagag gctcgtacgc tttgcccgag
1201 atgccaagcc acccactggc cccagccctg ccaccccggc tcgccgcagg ctgggcctgc
1261 gcagatccga cagaactgac atgcagagga taggagatgt gttgggcagc agtgaggact
1321 tcaggcggac agagtccacg ccggctggta gcgagaacac taaggacatt cggctgtagg
1381 agcagaacac ttcagcctgt gcaggtttat attgggaagc tgtagaggac caggacttgt
1441 gcagacgcca cagtctcccc agatatggac catcagtgac tcatgctgga tgaccccatg
1501 ctccgtcatt tgacaggggc tcaggatatt cactctgtgg tccagagtca actgttccca
1561 taacccctag tcatcgtttg tgtgtataag ttgggggaat taagtttcaa gaaaggcaag
1621 agctcaaggt caatgacacc cctggcctga ctcccatgca agtagctggc tgtactgcca
1681 aggtacctag gttggagtcc agcctaatca agtcaaatgg agaaacaggc ccagagagga
1741 aggtggctta ccaagatcac ataccagagt ctggagctga gctacctggg gtgggggcca
1801 agtcacaggt tggccagaaa accctggtaa gtaatgaggg ctgagtttgc acagtggtct
1861 ggaatggact gggtgccacg gtggacttag ctctgaggag taccccccagc ccaagagatg
1921 aacatctggg gactaatatc atagacccat ctggaggctc ccatgggcta ggagcagtgt
1981 gaggctgtaa cttatactaa aggttgtgtt gcctgctaaa aaaaa
```

18. **SEQ ID NO:18 The following sequence for the P2Y2 receptor is derived from H.sapiens epithelial cells (accession number U07225) protein sequence.**

```
MAADLGPWNDTINGTWDGDELGYRCRFNEDFKYVLLPVSYGVVC
VLGLCLNAVALYIFLCRLKTWNASTTYMFHLAVSDALYAASLPLLVYYYARGDHWPFS
TVLCKLVRFLFYTNLYCSILFLTCISVHRCLGVLRPLRSLRWGRARYARRVAGAVWVL
VLACQAPVLYFVTTSARGGRVTCHDTSAPELFSRFVAYSSVMLGLLFAVPFAVILVCY
VLMARRLLKPAYGTSGGLPRAKRKSVRTIAVVLAVFALCFLPFHVTRTLYYSFRSLDL
SCHTLNAINMAYKVTRPLASANSCLDPVLYFLAGQRLVRFARDAKPPTGPSPATPARR
RLGLRRSDRTDMQRIGDVLGSSEDFRRTESTPAGSENTKDIRL
```

19. **SEQ ID NO:19 The following sequence for the P2y3 receptor is derived from chick brain (accession number X98283).**

```
   1 ggcgcttcac ccagtaaaga gggaccatga gcatggccaa cttcacgggg gggaggaact
  61 cgtgcacctt ccatgaggaa ttcaagcagg tcctgctgcc cctggtctac tcagtggtgt
 121 tcctactggg gctgccactc aatgccgttg tcattgggca gatctggctg gcccgcaagg
 181 cgttgacccg caccaccatc tacatgctga acctggccat ggccgacctg ctttatgtct
 241 gctccctccc tctcctcatc tacaactaca cccagaagga ttactggccc tttggggact
 301 tcacctgcaa attcgtccgc ttccagttct acaccaacct gcacggcagc atcctcttcc
 361 tcacctgcat cagcgtccag cgctacatgg ggatctgcca ccccttggcc tcgtggcaca
 421 aaaagaaggg aaagaagctg acgtggctgg tgtgtgctgc cgtgtggttc atcgtcatcg
 481 cccagtgcct gcccaccttt gtcttcgcct ccaccggcac gcagaggaat cgcactgtct
 541 gctatgacct gagccccccg gaccgctcca catcctactt cccctatggc atcacgttga
 601 ccatcactgg cttcctgctg cccttcgcag ccatcctggc ctgctactgc agcatggccc
 661 gcatcctgtg ccagaaagac gagctgattg gcttggcggt gcacaagaag aaggacaagg
 721 ccgtgcgcat gatcatcatc gttgtcatcg tcttctccat cagcttcttc cccttccacc
 781 tcaccaagac catctacctg atcgtccgct cctcagccag cttgccctgc cctaccctgc
 841 aggcttttgc cattgcctac aagtgcacgc ggcctttgc cagcatgaac agcgtcctcg
 901 accccatcct cttctacttc acccagcgca agtttcgtga gagcacccgc tatctcctgg
 961 acaagatgag ctccaagtgg cggcaagacc actgcatcag ctacggctcc taggtggacg
1021 aggccacctc ggtgtcaccg gggctgggca tggagcaatt tgggttgaag ctgcatggtg
```

```
1081 cggagatggg gatgagccca gagtgctgcg ggtgccccat ctctggaggt gttggagatt
1141 agattggatg gggctctggg ccc
```

20. SEQ ID NO:20 The following sequence for the P2y3 receptor is derived from chick brain (accession number X98283) protein sequence.

```
MSMANFTGGRNSCTFHEEFKQVLLPLVYSVVFLLGLPLNAVVIG
QIWLARKALTRTTIYMLNLAMADLLYVCSLPLLIYNYTQKDYWPFGDFTCKFVRFQFY
TNLHGSILFLTCISVQRYMGICHPLASWHKKKGKKLTWLVCAAVWFIVIAQCLPTFVF
ASTGTQRNRTVCYDLSPPDRSTSYFPYGITLTITGFLLPFAAILACYCSMARILCQKD
ELIGLAVHKKKDKAVRMIIIVVIVFSISFFPFHLTKTIYLIVRSSASLPCPTLQAFAI
AYKCTRPFASMNSVLDPILFYFTQRKFRESTRYLLDKMSSKWRQDHCISYGS
```

21. SEQ ID NO:21 The following sequence for the P2Y4 receptor is derived from H.sapiens (accession number X91852). Other sequences have been published for P2Y4 receptors from human chromosome X (accession number U40223), and rat heart (accession number Y14705).

```
   1 aagggagctt gggtaggggc caggctagcc tgagtgcacc cagatgcgct tctgtcagct
  61 ctccctagtg cttcaaccac tgctctccct gctctacttt ttttgctcca gctcagggat
 121 gggggtgggc agggaaatcc tgccaccctc acttctcccc ttcccatctc caggggggcc
 181 atggccagta cagagtcctc cctgttgaga tccctaggcc tcagcccagg tcctggcagc
 241 agtgaggtgg agctggactg ttggtttgat gaggatttca agttcatcct gctgcctgtg
 301 agctatgcag ttgtctttgt gctgggcttg ggccttaacg ccccaaccct atggctcttc
 361 atcttccgcc tccgaccctg ggatgcaacg gccacctaca tgttccacct ggcattgtca
 421 gacaccttgt atgtgctgtc gctgcccacc ctcatctact attatgcagc ccacaaccac
 481 tggccctttg gcactgagat ctgcaagttc gtccgctttc ttttctattg gaacctctac
 541 tgcagtgtcc ttttcctcac ctgcatcagc gtgcaccgct acctgggcat ctgccaccca
 601 cttcgggcac tacgctgggg ccgccctcgc ctcgcaggcc ttctctgcct ggcagtttgg
 661 ttggtcgtag ccggctgcct cgtgcccaac ctgttctttg tcacaaccag caacaaaggg
 721 accaccgtcc tgtgccatga caccactcgg cctgaagagt ttgaccacta tgtgcacttc
 781 agctcggcgg tcatggggct gctctttggc gtgccctgcc tggtcactct tgtttgctat
 841 ggactcatgg ctcgtcgcct gtatcagccc ttgccaggct ctgcacagtc gtcttctcgc
 901 ctccgctctc tccgcaccat agctgtggtg ctgactgtct ttgctgtctg cttcgtgcct
 961 ttccacatca cccgcaccat ttactacctg gccaggctgt tggaagctga ctgccgagta
1021 ctgaacattg tcaacgtggt ctataaagtg actcggcccc tggccagtgc caacagctgc
1081 ctggatcctg tgctctactt gctcactggg gacaaatatc gacgtcagct ccgtcagctc
1141 tgtggtggtg gcaagcccca gccccgcacg gctgcctctt ccctggcact agtgtccctg
1201 cctgaggata gcagctgcag gtgggcggcc acccccccagg acagtagctg ctctactcct
1261 agggcagata gattgtaaca cgggaagccg ggaagtgaga gaaaagggga tgagtgcagg
1321 gcagaggtga gggaacccaa tagtgatacc tggtaaggtg cttcttcctc ttttccaggc
1381 tctggagaga agccctcacc ctgagggttg ccagggaggc agggatatc
```

22. SEQ ID NO:22 The following sequence for the P2Y4 receptor is derived from H.sapiens (accession number X91852) Protein sequence.

```
MASTESSLLRSLGLSPGPGSSEVELDCWFDEDFKFILLPVSYAV
VFVLGLGLNAPTLWLFIFRLRPWDATATYMFHLALSDTLYVLSLPTLIYYYAAHNHWP
FGTEICKFVRFLFYWNLYCSVLFLTCISVHRYLGICHPLRALRWGRPRLAGLLCLAVW
LVVAGCLVPNLFFVTTSNKGTTVLCHDTTRPEEFDHYVHFSSAVMGLLFGVPCLVTLV
CYGLMARRLYQPLPGSAQSSSRLRSLRTIAVVLTVFAVCFVPFHITRTIYYLARLLEA
DCRVLNIVNVVYKVTRPLASANSCLDPVLYLLTGDKYRRQLRQLCGGGKPQPRTAASS
LALVSLPEDSSCRWAATPQDSSCSTPRADRL
```

23. SEQ ID NO:23 The following sequence for the P2Y6 receptor is derived from H.sapiens placenta (accession numberX97058). Other sequences have been published for P2Y6 receptors from human placenta (accession number AF007893), and human activated T-cells (accession number U52464).

```
   1 ctcagtttcc tcatctgctg cctctccaga cttctgccag aacattgcac gcgacagttt
  61 caggcacaga actgactggc agcaggggct gctccacgag tgggaatttg ctccagcact
 121 tcacggactg caagcgaggc acttgctaac tcttggataa caagacctct gccagaagaa
 181 ccatggcttt ggaaggcgga gttcaggctg aggagatggg tgcggtcctc agtgagcccc
 241 tgcctccctg aacataggaa acccacctgg gcagccatgg aatgggacaa tggcacaggc
 301 caggctctgg gcttgccacc caccacctgt gtctaccgcg agaacttcaa gcaactgctg
 361 ctgccacctg tgtattcggc ggtgctggcg gctggcctgc cgctgaacat ctgtgtcatt
 421 acccagatct gcacgtcccg ccgggccctg acccgcacgg ccgtgtacac cctaaacctt
 481 gctctggctg acctgctata tgcctgctcc ctgcccctgc tcatctacaa ctatgcccaa
 541 ggtgatcact ggccctttgg cgacttcgcc tgccgcctgg tccgcttcct cttctatgcc
 601 aacctgcacg gcagcatcct cttcctcacc tgcatcagct tccagcgcta cctgggcatc
 661 tgccacccgc tggccccctg gcacaaacgt gggggccgcc gggctgcctg gctagtgtgt
 721 gtagccgtgt ggctggccgt gacaacccag tgcctgccca cagccatctt cgctgccaca
 781 ggcatccagc gtaaccgcac tgtctgctat gacctcagcc cgcctgccct ggccacccac
 841 tatatgccct atggcatggc tctcactgtc atcggcttcc tgctgccctt tgctgccctg
 901 ctggcctgct actgtctcct ggcctgccgc ctgtgccgcc aggatggccc ggcagagcct
 961 gtggcccagg agcggcgtgg caaggcggcc cgcatggccg tggtggtggc tgctgccttt
1021 gccatcagct tcctgccttt tcacatcacc aagacagcct acctggcagt gcgctcgacg
1081 ccgggcgtcc cctgcactgt attggaggcc tttgcagcgg cctacaaagg cacgcggccg
1141 tttgccagtg ccaacagcgt gctggacccc atcctcttct acttcaccca gaagaagttc
1201 cgccggcgac cacatgagct cctacagaaa ctcacagcca aatggcagag gcagggtcgc
1261 tgagtcctcc aggtcctggg cagccttcat atttgccatt gtgtccgggg caccaggagc
1321 cccaccaacc ccaaaccatg cggagaatta gagttcagct cagctgggca tggagttaag
1381 atccctcaca ggacccagaa gctcaccaaa aactatttct tcagcccctt ctctggccca
1441 gaccctgtgg gcatggagat ggacagacct gggcctggct cttgagaggt cccagtcagc
1501 catggagagc tggggaaacc acattaaggt gctcacaaaa atacagtgtg acgtgtactg
1561 tcaaaaaaaa a
```

24. SEQ ID NO:22 The following sequence for the P2Y6 receptor is derived from H.sapiens (accession number X91852) Protein sequence.

```
MEWDNGTGQALGLPPTTCVYRENFKQLLLPPVYSAVLAAGLPLNICVITQICTSRRALTRTA
VYTLNLALADLLYACSLPLLIYNYAQGDHWPFGDFACRLVRFLFYANLHGSILFLTCISFQR
YLGICHPLAPWHKRGGRRAAWLVCVAVWLAVTTQCLPTAIFAATGIQRNRTVCYDLSPPALA
THYMPYGMALTVIGFLLPFAALLACYCLLACRLCRQDGPAEPVAQERRGKAARMAVVVAAAF
AISFLPFHITKTAYLAVRSTPGVPCTVLEAFAAAYKGTRPFASANSVLDPILFYFTQKKFRR
RPHELLQKLTAKWQRQGR
```

25. SEQ ID NO:24 The following sequence for the P2Y11 receptor is derived from human placenta (accession number AF030335). Other sequences have been published for P2Y11 receptors from human HL-60 cells (accession number AJ298334).

```
   1 atggatcgag gtgccaagtc ctgccctgcc aacttcttgg cagctgccga cgacaaactc
  61 agtgggttcc agggggactt cctgtggccc atactggtgg ttgagttcct ggtggccgtg
 121 gccagcaatg gcctggccct gtaccgcttc agcatccgga agcagcgccc atggcacccc
 181 gccgtggtct tctctgtcca gctggcagtc agcgacctgc tctgcgctct gacgctgccc
 241 ccgctggccg cctacctcta tccccccaag cactggcgct atggggaggc cgcgtgccgc
 301 ctggagcgct tcctcttcac ctgcaacctg ctgggcagcg tcatcttcat cacctgcatc
 361 agcctcaacc gctacctggg catcgtgcac cccttcttcg cccgaagcca cctgcgaccc
 421 aagcacgcct gggccgtgag cgctgccggc tgggtcctgg ccgccctgct ggccatgccc
 481 acactcagct tctcccacct gaagaggccg cagcaggggg cgggcaactg cagcgtggcc
 541 aggcccgagg cctgcatcaa gtgtctgggg acagcagacc acgggctggc ggcctacaga
 601 gcgtatagcc tggtgctggc ggggttgggc tgcggcctgc cgctgctgct cacgctggca
 661 gcctacggcg ccctcgggcg ggccgtgcta cgcagcccag gcatgactgt ggccgagaag
 721 ctgcgtgtgg cagcgttggt ggccagtggt gtggccctct acgccagctc ctatgtgccc
 781 taccacatca tgcgggtgct caacgtggat gctcggcggc gctggagcac ccgctgcccg
 841 agctttgcag acatagccca ggccacagca gccctggagc tggggcccta cgtgggctac
 901 caggtgatgc ggggcctcat gcccctggcc ttctgtgtcc accctctact ctacatggcc
 961 gcagtgccca gcctgggctg ctgctgccga cactgccccg gctacaggga cagctggaac
1021 ccagaggacg ccaagagcac tggccaagcc ctgcccctca atgccacagc cgcccctaaa
1081 ccgtcagagc cccagtcccg tgagctgagc caatga
```

26. The following sequence for the P2Y11 receptor is derived from human placenta (accession number AF030335) protein sequence.

MDRGAKSCPANFLAAADDKLSGFQGDFLWPILVVEFLVAVASNG
LALYRFSIPKQRPWHPAVVFSVQLAVSDLLCALTLPPLAAYLYPPKHWRYGEAACRLE
RFLFTCNLLGSVIFITCISLNRYLGIVHPFFARSHLRPKHAWAVSAAGWVLAALLAMP
TLSFSHLKRPQQGAGNCSVARPEACIKCLGTADHGLAAYRAYSLVLAGLGCGLPLLLT
LAAYGALGRAVLRSPGMTVAEKLRVAALVASGVALYASSYVPYHIMRVLNVDARRRWS
TRCPSFADIAQATAALELGPYVGYQVMRGLMPLAFCVHPLLYMAAVPSLGCCCRHCPG
YRDSWNPEDAKSTGQALPLNATAAPKPSEPQSRELSQ

VII. CLAIMS

What is claimed is:

1. A method of modulating odor sensitivity in a subject, comprising administering a composition to the subject, wherein the composition is an agonist of a P2X or P2Y purinergic receptor.
2. The method of claim 1, wherein the P2X purinergic receptor is a $P2X_1$, $P2X_3$, $P2X_4$, $P2X_5$, or $P2X_6$ purinergic receptor.
3. The method of claim 1, wherein the P2Y purinergic receptor is a $P2Y_1$, $P2Y_2$, $P2Y_4$, $P2Y_6$, or $P2Y_{11}$ purinergic receptor.
4. The method of claim 1, wherein the concentration of the composition is less than or equal to 30 μM, 20 μM, 10 μM, 5 μM, 4 μM, 3 μM, 2 μM, 1.6 μM, 1 μM, 0.5 μM, 0.1 μM, 0.05 μM, or 0.01 μM.
5. The method of claim 1, wherein the agonist is a P2X selective agonist.
6. The method of claim 5, wherein the P2X selective agonist is α,β-meATP, β,γ-meATP, BzATP
7. The method of claim 1 or 3, wherein the agonist is a P2Y selective agonist.
8. The method of claim 7, wherein the P2Y selective agonist is ADP, UTP, UTPγS, UDP, 2Cl-ADP, 2MeSADP, ADPβS, ADPβF.
9. The method of claim 1, wherein the agonist is a non-selective agonist.
10. The method of claim 9, wherein the non-selective agonist is ATP, ATPγS, 2MeSATP, Ap_4A.
11. The method of claim 1, wherein the agonist enhances the Ca^{2+} released from coapplication of an odor stimulant and the agonist
12. The composition of claim 11, wherein the agonist is βγ-methylene ATP.
13. The method of claim 1, wherein the agonist suppresses the Ca^{2+} released from the coapplication of an odor stimulant and the agonist.
14. The method of claim 13, wherein the agonist is UTP and ADP-βS.
15. The method of claim 1, wherein the agonist increases the ratio of observed co-application-evoked calcium transient over the sum of individual odor and P2 agonists peak amplitudes in a cell activation assay.
16. The method of claim 1, wherein the agonist decreases the ratio of observed co-application evoked calcium transient over the sum of individual odor and P2 agonists peak amplitudes in a cell activation assay.
17. The method of claim 15 or 16, wherein the ratio is 0.69 to .83.
18. The method of claim 17, wherein the agonist is ATP.

19. The method of claims 15 or 16, wherein the ratio is 0.72 to .92.

20. The method of claim 19, wherein the agonist is βγ-methylene ATP.

21. The method of claims 15 or 16, wherein the ratio is 0.52 to .64.

22. The method of claim 21, wherein the agonist is ADP-βS.

23. A method of modulating odor sensitivity in a subject, comprising administering a composition to the subject, wherein the composition is an agonist of a P2X purinergic receptor.

24. A method of modulating odor sensitivity in a subject, comprising administering a composition to the subject, wherein the composition is an agonist of a P2Y purinergic receptor.

25. A method of screening for an agonist or antagonist of a purinergic receptor of the olfactory system, comprising

(a) contacting a purinergic receptor with a test compound;

(b) detecting intracellular calcium levels; and

(c) screening for a change in calcium levels as compared to a control level, wherein a change in the calcium level relative to a control indicates the compound is an agonist or an antagonist of a purinergic receptor of the olfactory system.

26. The method of claim 25, wherein the compound is an agonist.

27. The method of claim 25, wherein the compound is an antagonist.

28. The method of claim 25, wherein the change in calcium levels is transient.

29. The method of claim 25, wherein the change in calcium levels is sustained.

30. The method of claims 25, wherein the compound is selected when the calcium level increases as compared to a control level.

31. The method of claim 25, wherein compound is selected when the calcium level decreases as compared to a control level.

32. The method of claim 25, wherein the purinergic receptor is a P2X or a P2Y purinergic receptor.

33. The method of claim 25, wherein the P2X purinergic receptor is a $P2X_1$, $P2X_3$, $P2X_4$, $P2X_5$, or $P2X_6$ purinergic receptor.

34. The method of claim 25, wherein the P2Y purinergic receptor is a $P2Y_1$, $P2Y_2$, $P2Y_4$, $P2Y_6$, or $P2Y_{11}$ purinergic receptor.

35. The method of claim 25, further comprising the step of:

(d) screening for reversibility of response by removing the agonist or antagonist during the assay.

36. The method of claim 35, further comprising the step of:

(e) screening for dependence upon extracellular Ca^{2+} by repeating the assay in a solution devoid of extracellular Ca^{2+}.

37. The method of claim 25, wherein the purinergic receptor is on olfactory epithelium.

38. The method of claim 35, wherein the olfactory epithelium comprises olfactory receptor neurons.

39. The method of claim 35, wherein the olfactory epithelium comprises sustentacular cells.

40. The method of claim 25, wherein the calcium levels are detected using a calcium indicator.

41. The method of claim 35, wherein the calcium indicator is Fluo-4 AM.

42. The method of claim 35, wherein the calcium indicator is Fura-2/AM.

43. The method of claim 35, wherein the calcium indicator is Indo-1.

44. The method of claim 35, wherein the calcium indicator is or Indo-4.

45. A method of screening for an agonist of a purinergic receptor of the olfactory system, comprising

(a) contacting a purinergic receptor with a test compound;

(b) detecting intracellular calcium levels; and

(c) screening for a change in calcium levels as compared to a control level, wherein a change in the calcium level relative to a control indicates the compound is an agonist of a purinergic receptor of the olfactory system.

46. A method of screening for an antagonist of a purinergic receptor of the olfactory system, comprising

(a) contacting a purinergic receptor with a test compound;

(b) detecting intracellular calcium levels; and

(c) screening for a change in calcium levels as compared to a control level, wherein a change in the calcium level relative to a control indicates the compound is an antagonist of a purinergic receptor of the olfactory system.

47. A method of screening for an agonist or an antagonist of a purinergic receptor of the olfactory system, comprising

(a) contacting a first purinergic receptor cell with a set of test compounds ;

(b) detecting calcium levels in the first purinergic receptor cell; and

(c) selecting each compound in the set that contacted the first purinergic receptor cell, wherein the first purinergic receptor cell showed a transient change in calcium as compared to a control level, indicating the compound is an agonist or an antagonist of a purinergic receptor of the olfactory system.

48. The method of claim 35, further comprising the step:

(d) contacting a second purinergic receptor cell with one test compound selected in step (c).

49. The method of claim 35, further comprising the step:

(e) detecting calcium levels in the second purinergic receptor cell, wherein a transient change in calcium as compared to a control level indicates the compound is an agonist or an antagonist of a purinergic receptor of the olfactory system.

50. A method of screening for an agonist or an antagonist of a purinergic receptor of the olfactory system, comprising

(a) contacting a test compound with a cell that expresses a heterologous nucleic acid that encodes a purinergic receptor; and

(b) detecting calcium levels in the cell;

a transient change in calcium as compared to a control level, indicating an agonist or an

antagonist of a purinergic receptor of the olfactory system..

51. A method of modulating odor sensitivity in a subject, comprising administering a composition to the subject, wherein the composition is an antagonist of a P2X or P2Y purinergic receptor.

52. The method of claim 51, wherein the P2X purinergic receptor is a $P2X_1$, $P2X_3$, $P2X_4$, $P2X_5$, or $P2X_6$ purinergic receptor.

53. The method of claim 51, wherein the P2Y purinergic receptor is a $P2Y_1$, $P2Y_2$, $P2Y_4$, $P2Y_6$, or $P2Y_{11}$ purinergic receptor.

54. The method of claim 51, wherein the odor sensitivity of the subject is increased.

55. The method of claim 51, wherein the antagonist is a P2X selective antagonist.

56. The method of claim 55, wherein the P2X selective antagonist is NF023, NF279 or KN-62a.

57. The method of claim 51, wherein the antagonist is a P2Y selective antagonist.

58. The method of claim 55, wherein the antagonist is ARL 67085, FPL 66096, A3P5PS, MRS 2179, 2-hexylthio-ATP, or 2-cyclohexylthio-ATP.

59. The method of claim 51, wherein the antagonist is a non-selective antagonist.

60. The method of claim 55, wherein the non-selective antagonist is Suramin, PPADS, Iso-PPADS, P5P, Reactive blue 2, Reactive Red, Trypan Blue, Evans Blue, or DIDS.

61. The method of claim 51, wherein the antagonist suppresses the Ca^{2+} released from coapplication of an odor stimulant and the antagonist.

62. The method of claim 51, wherein the agonist increases the ratio of observed co-application-evoked calcium transient over the sum of individual odor and P2 antagonists peak amplitudes in a cell activation assay.

63. A method of modulating odor sensitivity in a subject, comprising administering a composition to the subject, wherein the composition is an antagonist of a P2X purinergic receptor.

64. A method of modulating odor sensitivity in a subject, comprising administering a composition to the subject, wherein the composition is an antagonist of a P2Y purinergic receptor.

65. A method of protecting a cell from the effects of odor stimulation comprising administering a composition to the cell, wherein the composition is an antagonist or an agonist of a P2Y or a P2X purinergic receptor.

66. A method of modulating odor sensitivity in a subject, comprising inhibiting the interaction of ATP or ATP analog with a P2X or a P2Y purinergic receptor.

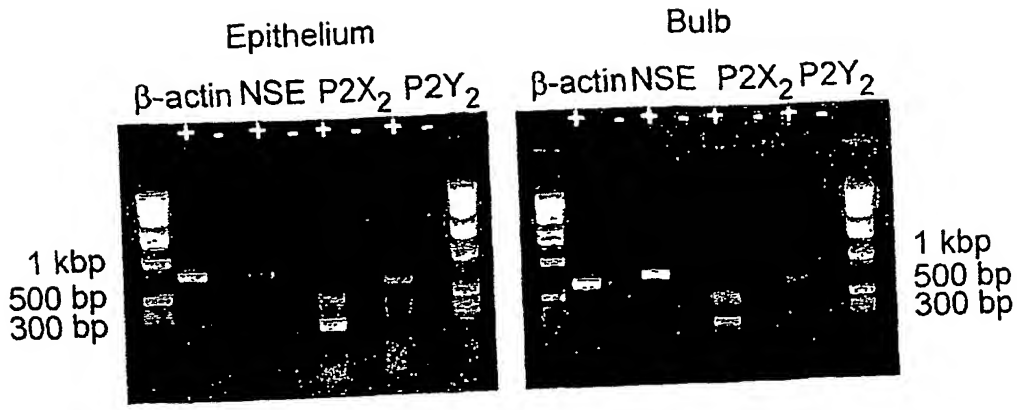


FIG.1A

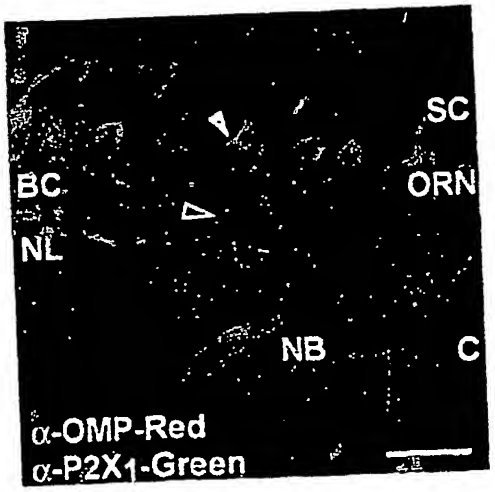


FIG.1B

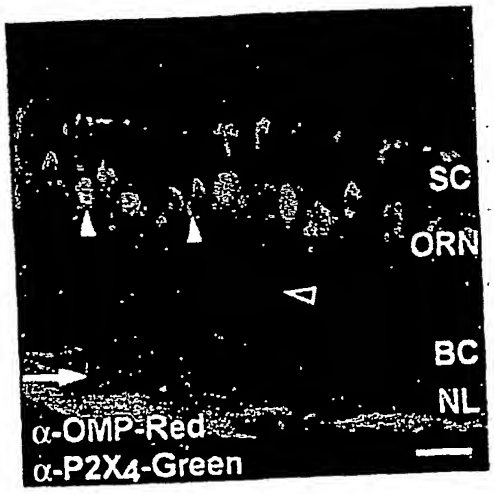
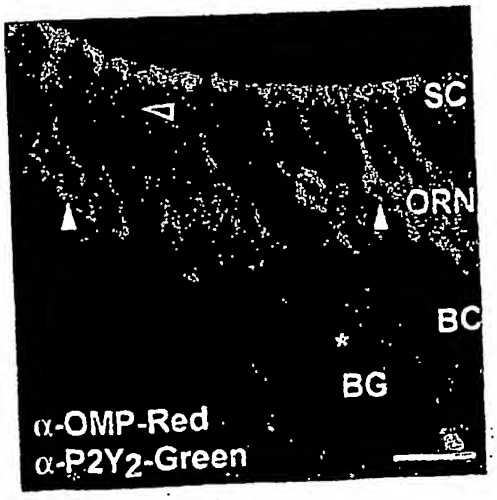


FIG.1C



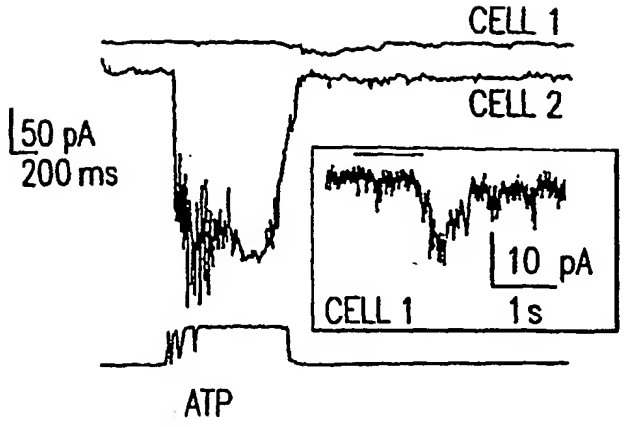


FIG.2A

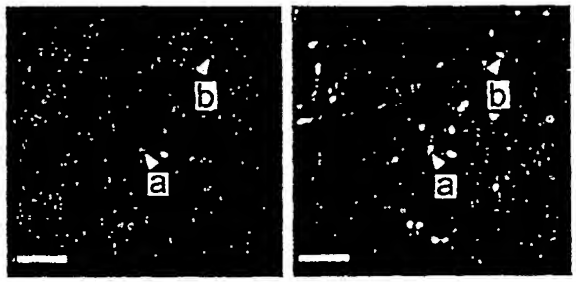


FIG.2B

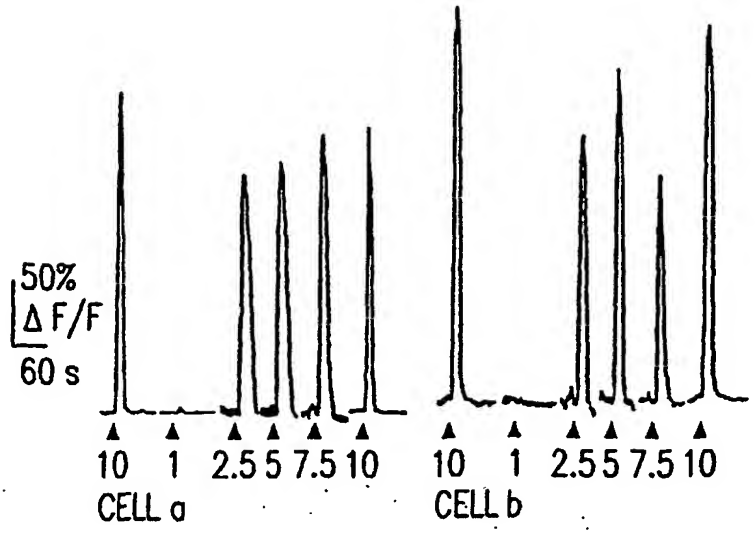


FIG.2C

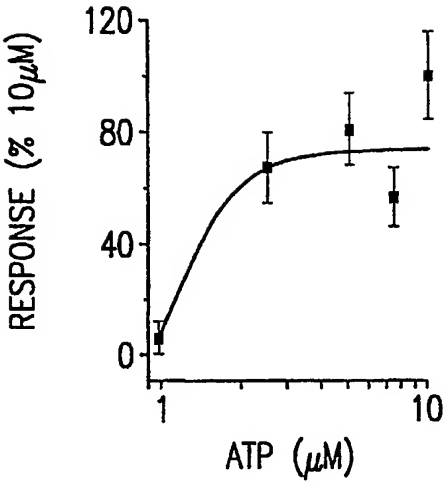


FIG.2D

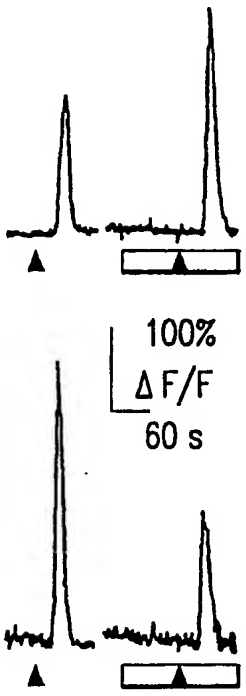


FIG.2E

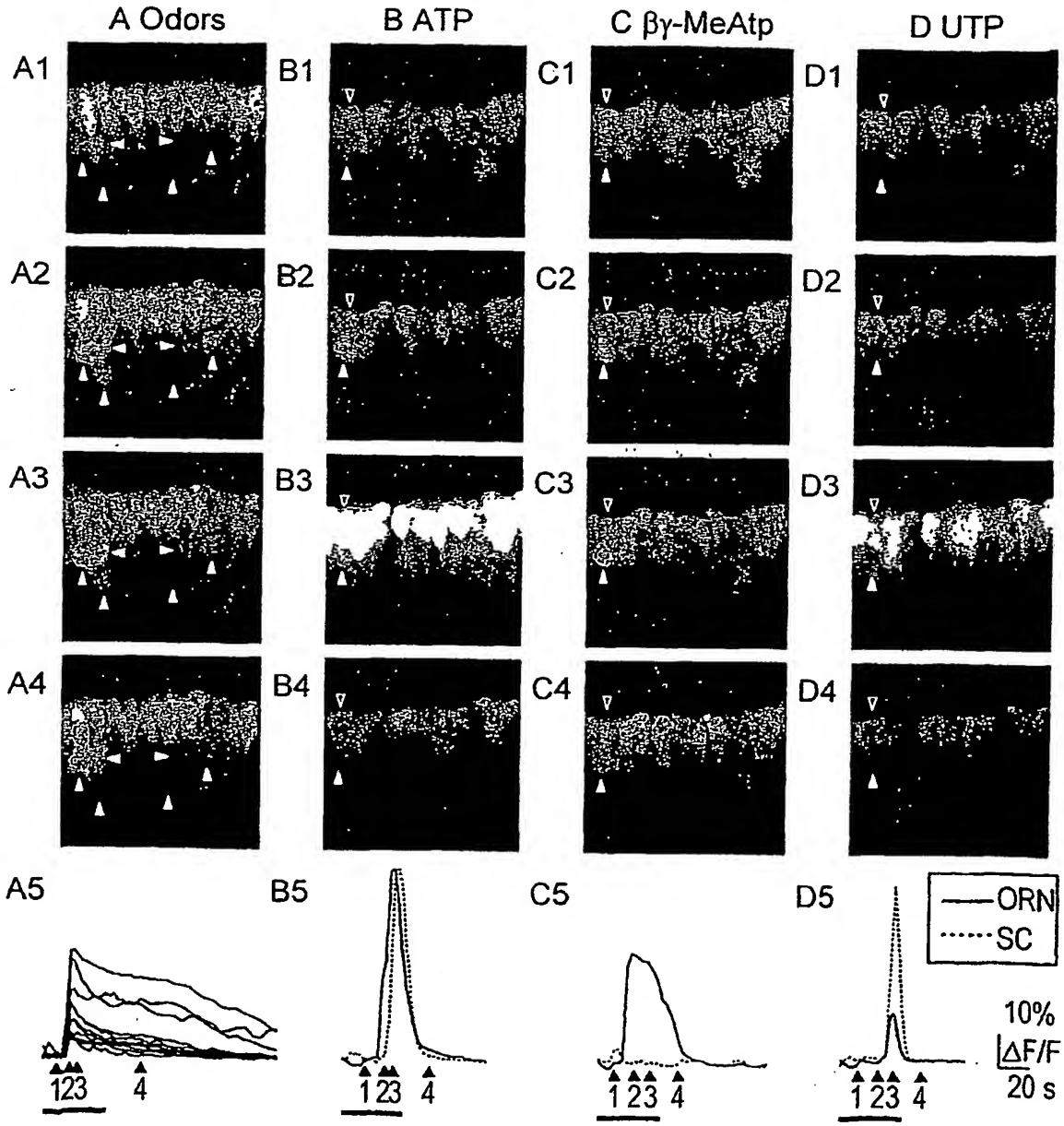

A Odors
B ATP
C βγ-MeAtp
D UTP
A1
B1
C1
D1
A2
B2
C2
D2
A3
B3
C3
D3
A4
B4
C4
D4
A5
B5
C5
D5
ORN
SC
10%
ΔF/F
20 s
1 2 3
4

FIG.3

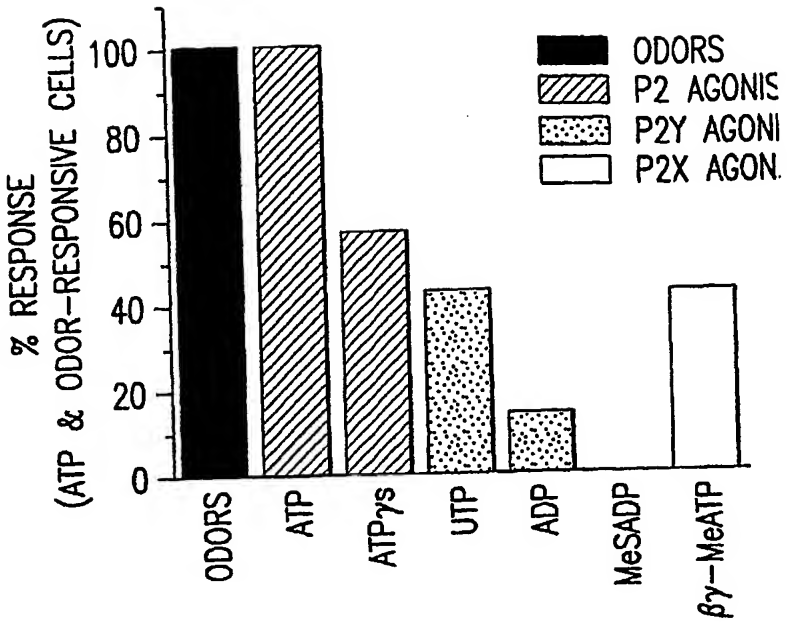

% RESPONSE
(ATP & ODOR-RESPONSIVE CELLS)
100
80
60
40
20
0
ODORS
P2 AGONIS
P2Y AGONI
P2X AGON.
ODORS
ATP
ATPγs
UTP
ADP
MeSADP
βγ-MeATP

FIG.4A

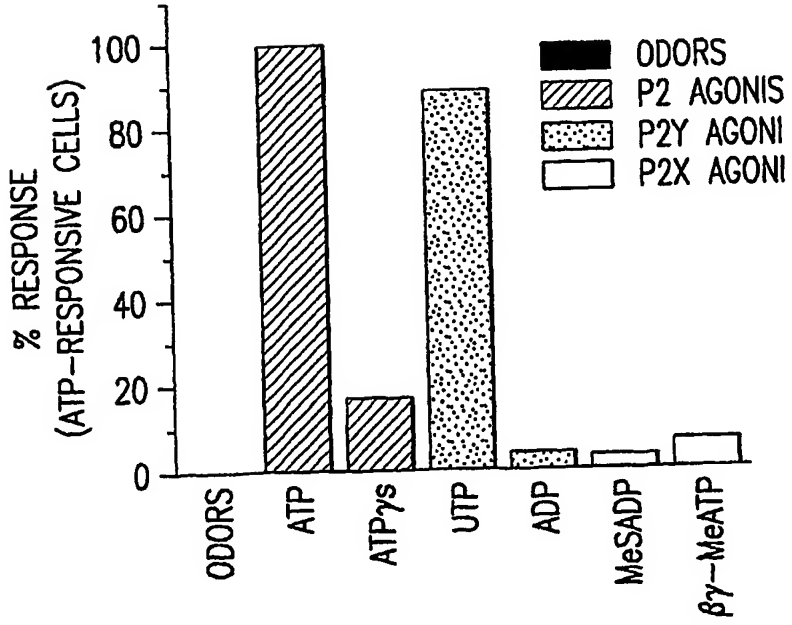

% RESPONSE
(ATP-RESPONSIVE CELLS)
100
80
60
40
20
0
ODORS
P2 AGONIS
P2Y AGONI
P2X AGONI
ODORS
ATP
ATPγs
UTP
ADP
MeSADP
βγ-MeATP

FIG.4B

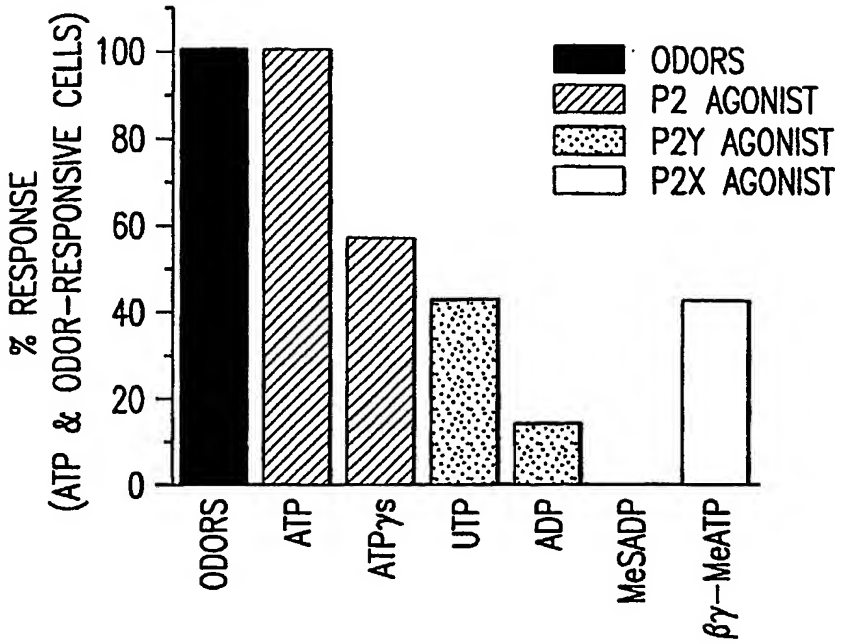

% RESPONSE
(ATP & ODOR-RESPONSIVE CELLS)
100
80
60
40
20
0
ODORS
P2 AGONIST
P2Y AGONIST
P2X AGONIST
ODORS
ATP
ATPγs
UTP
ADP
MeSADP
βγ-MeATP

FIG.4A

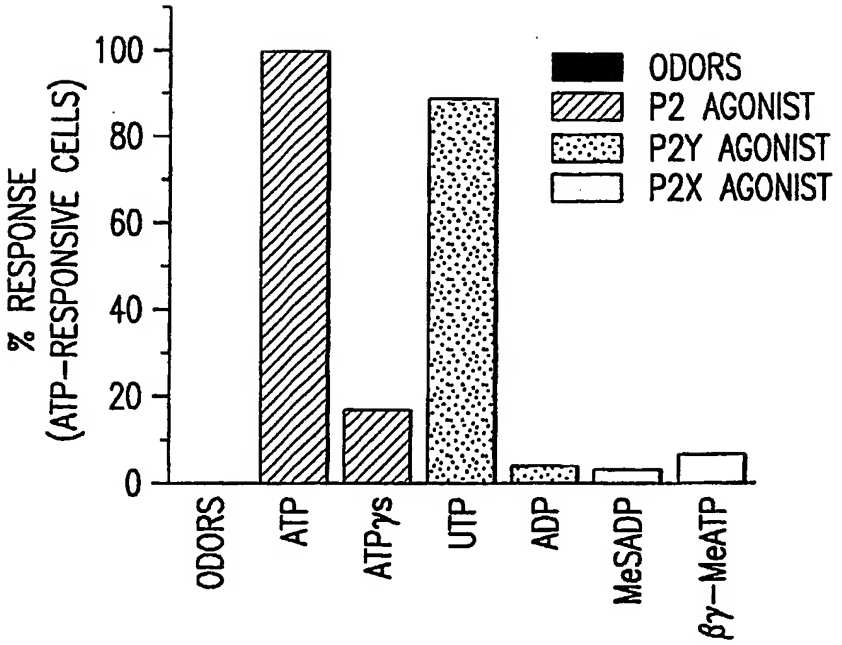

% RESPONSE
(ATP-RESPONSIVE CELLS)
100
80
60
40
20
0
ODORS
P2 AGONIST
P2Y AGONIST
P2X AGONIST
ODORS
ATP
ATPγs
UTP
ADP
MeSADP
βγ-MeATP

FIG.4B

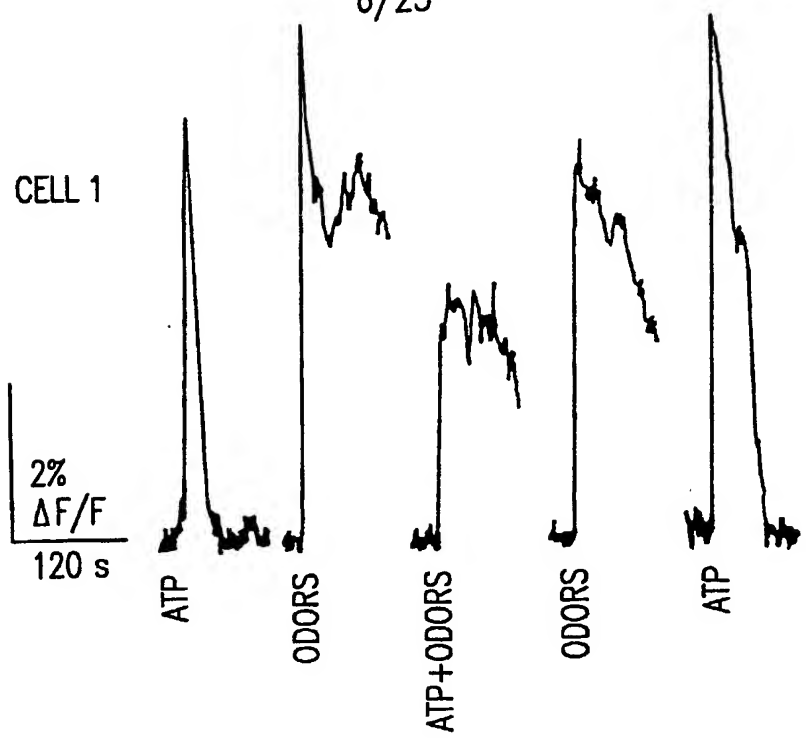

CELL 1
2%
ΔF/F
120 s
ATP
ODORS
ATP+ODORS
ODORS
ATP

FIG.5A

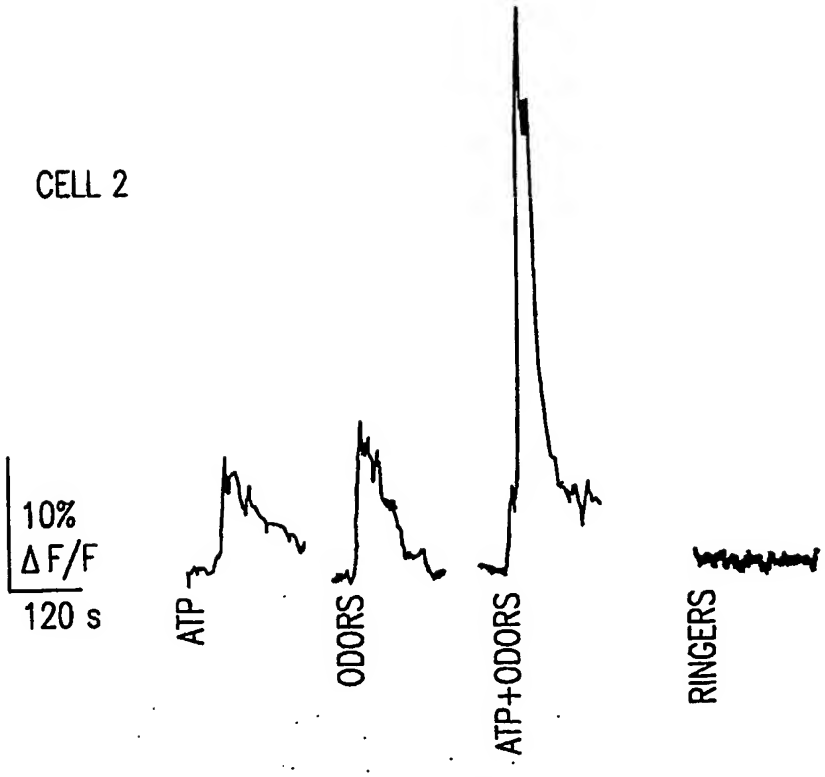

CELL 2
10%
ΔF/F
120 s
ATP
ODORS
ATP+ODORS
RINGERS

FIG.5B

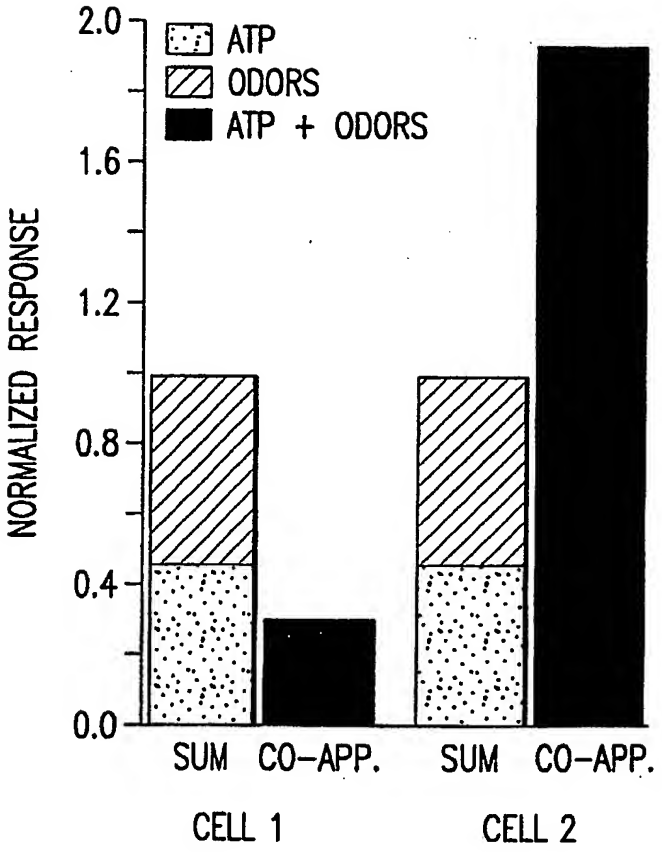

2.0
1.6
1.2
0.8
0.4
0.0
NORMALIZED RESPONSE
ATP
ODORS
ATP + ODORS
SUM
CO-APP.
SUM
CO-APP.
CELL 1
CELL 2

FIG.5C

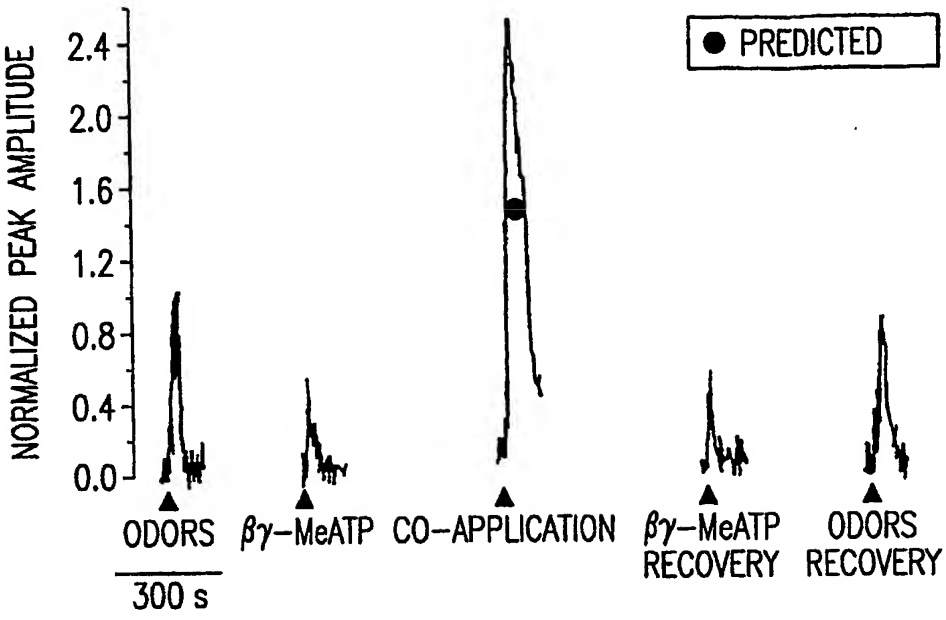

NORMALIZED PEAK AMPLITUDE
2.4
2.0
1.6
1.2
0.8
0.4
0.0
PREDICTED
ODORS
βγ–MeATP
CO–APPLICATION
βγ–MeATP RECOVERY
ODORS RECOVERY
300 s

FIG.6A

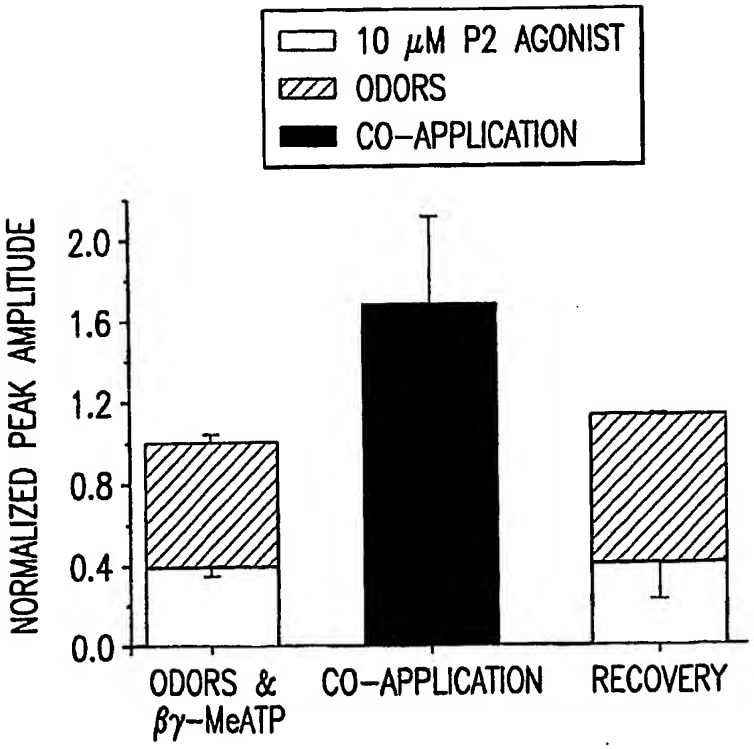

10 μM P2 AGONIST
ODORS
CO–APPLICATION
NORMALIZED PEAK AMPLITUDE
2.0
1.6
1.2
0.8
0.4
0.0
ODORS & βγ–MeATP
CO–APPLICATION
RECOVERY

FIG.6B

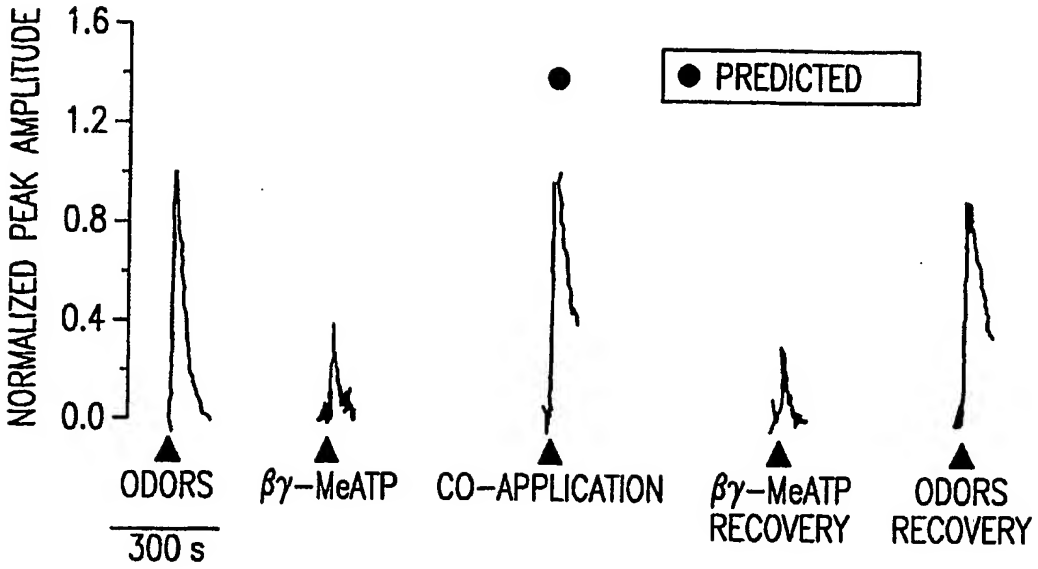

NORMALIZED PEAK AMPLITUDE
1.6
1.2
0.8
0.4
0.0
PREDICTED
ODORS
βγ–MeATP
CO–APPLICATION
βγ–MeATP RECOVERY
ODORS RECOVERY
300 s

FIG.6C

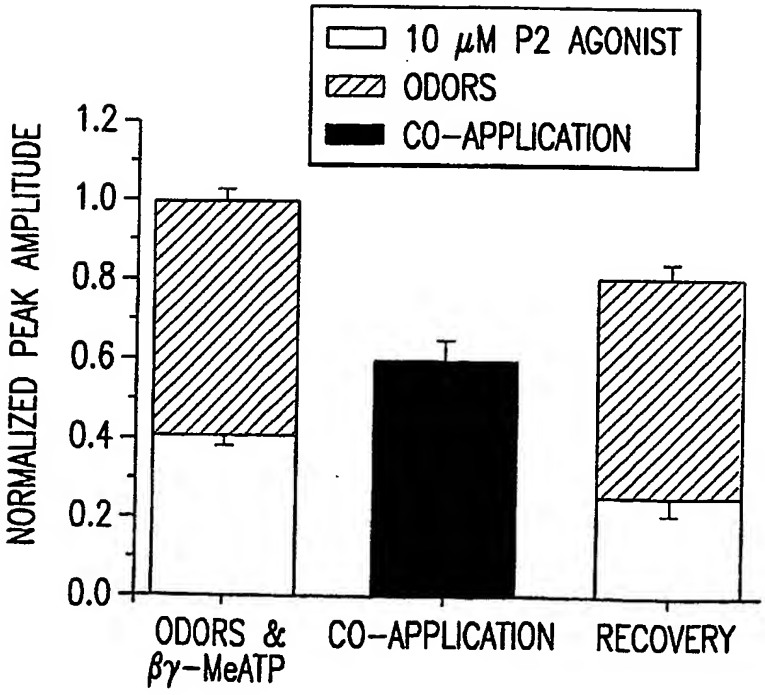

10 μM P2 AGONIST
ODORS
CO–APPLICATION
NORMALIZED PEAK AMPLITUDE
1.2
1.0
0.8
0.6
0.4
0.2
0.0
ODORS & βγ–MeATP
CO–APPLICATION
RECOVERY

FIG.6D

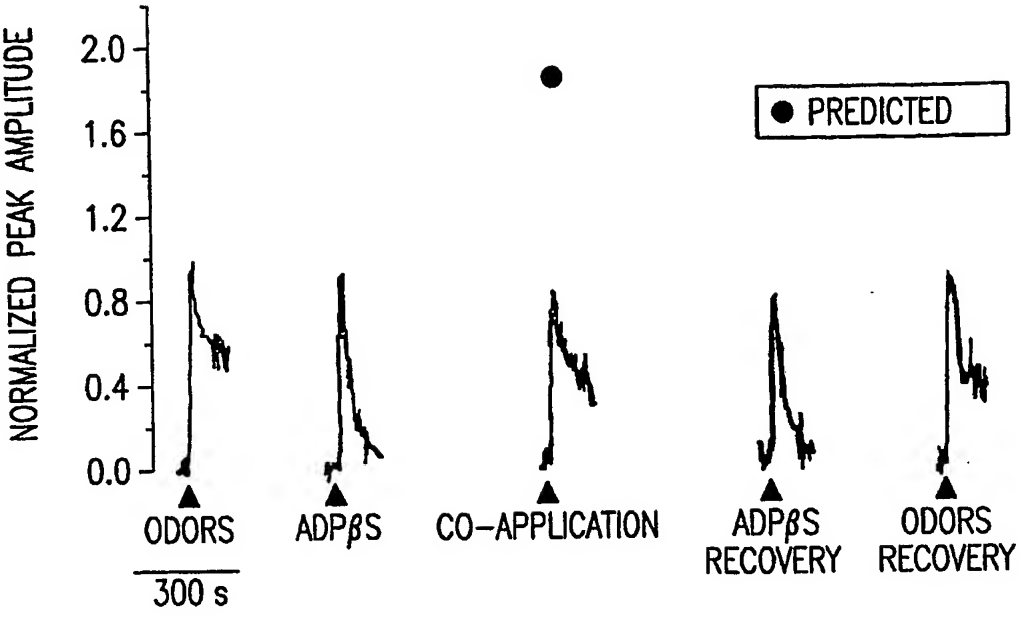

NORMALIZED PEAK AMPLITUDE
2.0
1.6
1.2
0.8
0.4
0.0
PREDICTED
ODORS
ADPβS
CO-APPLICATION
ADPβS RECOVERY
ODORS RECOVERY
300 s

FIG.6E

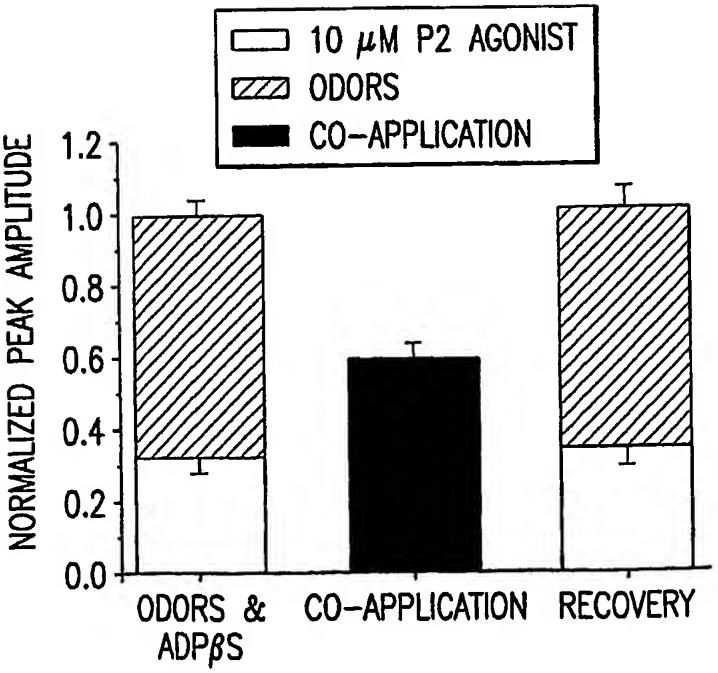

10 μM P2 AGONIST
ODORS
CO-APPLICATION
NORMALIZED PEAK AMPLITUDE
1.2
1.0
0.8
0.6
0.4
0.2
0.0
ODORS & ADPβS
CO-APPLICATION
RECOVERY

FIG.6F

M1
P2X1 -MARRLQDELSAFFEYDTPRMVLVRNKKVGVIFRLIQLVVLVVVIGWVFVYEKGYQTSS 59
P2X2 -MVRRLARGCWSAFWDYEIPKVIVVRNRRLGFVHRMVDLLILLYFVWYVFIVQKSYQDSE 59
P2X3 -------MNCISDFFTYETITKSVVVKSWTIGIINRAVQLLIISYFVGWVFLHEKAYQVRD 53
P2X4 --MAGCCSVLGSFLFEYDTPRIVLIRSRKVGLMNRAVQLLILAVVIGWVFVWEKGYQETD 58
P2X5 -MGQAAWKGFVLSLFDYKTPAKFVVAKSKKVGLLYRVLQIIILLVLIIWVFLIKKSYQDID 59
P2X6 MASAVAAALVSWGFLDYKTEKYVMTRNCWGISQRLLQLGVVVWVIGWALLAKKGYQEMD 60
* 118
P2X1 DLISSVSV-RLKGLAVTQLQGLGPQWMDVADYVFPAHGDSSFVVMTNFIVTPQQTQGHCA
P2X2 TGPESSIITKWKGLITMSEDKV-----WDVEEYMKPPEGGSVVSIIITRIEVTPSQTLGTCP 114
P2X3 TAIESSVTKWKGFG-----RYANRVMDVSDYMTPPQGTSVFVIIIKIIVTENQMQGFCP 108
P2X4 SVVSSVTT-KAKGVAVTNTSQLGFRIWDVADYMIPAQEENSLFIMTLWIVTVNQTQSTCP 117
P2X5 TSLQSAVVTKWKGVAYTNTTMLGERLWDVADFMIPSQGENVFFVVTNLIVTPNQRQGICA 119
P2X6 MDPQISVITKLKGVSVTQWKELEKRLWDVADFVRPSQGENVFFLVTNFLVTPAQVQGRCP 120
* * * * * *
P2X1 ENPE--GGICQDDSGCTPGKAERKAQGIRTGKCVPFNGTVK-TCEIFGWCPVEVDDKIPS 175
P2X2 ESMRVHSSTCHSDDDCIAGQLDMQGNGIRTGHCVPYYHGDSKTCEVSAWCPVEDG-TSDN 173
P2X3 ENEE--KYRCVSDSQCGPER--FPGGGILTGRCVNYSSVLR-TCEIQGWCPTEVD-TVEM 162
P2X4 EIPD-KTSICNSDADCTPGLRDTHSSGVAIGRCVPFNESVK-TCEVAAWCPVENDVGVPT 175
P2X5 EREGIPDGECSEDDDCHAGESVVAGHGLKTGRCLRVGNSTRGTCEIFAWCPVETK-SMPT 178
P2X6 EHPSVPLANGWADEDCPEGEMGTYSHGIKTGACVAFNGTHR-TCEIWSWCPVESS-AVPR 178
* * *
P2X1 PALLREAENFTLFIKNSISFPRFKVNRRNLVEEVNGTYMKKCLYHKIQHPLCPVFNLGYV 235
P2X2 HFLGKMAPNFTILIKNSIHYPKFKFSKGNIASQKSDYLKHLQTFDQDSDPYCPIFRLGFI 232
P2X3 P-IMMEAENFTIIFIKNSIRFPLFNFEKGNLLPNLTDKDIKRCRFHPEKAPFCPILRVGDV 221
P2X4 PAFLKAAENFTLLVKNNIWYPKFNFSKRNILPNITTSYLKSDIYNAQTDPFCPIFRLGTI 235
P2X5 DPLLKDAESFTISIKNFIRFPKFNFSKANVLETDNKHFLKTDHF-SSTNLYCPIFRLGSI 237
P2X6 KPLLAQAKNFTLFIKNTVTFNKFNFSRTNALDTWDNTYFKYQLYDSLSSPYCPVFRIGDL 238

FIG. 7A

(H5) M2
P2X1 292
P2X2 290
P2X3 281
P2X4 295
P2X5 296
P2X6 289
P2X1 351
P2X2 350
P2X3 341
P2X4 355
P2X5 356
P2X6 349
P2X1 399
P2X2 410
P2X3 397
P2X4 388
P2X5 416
P2X6 379
P2X1 399
P2X2 472
P2X3 397
P2X4 388
P2X5 455
P2X6 379

FIG. 7B

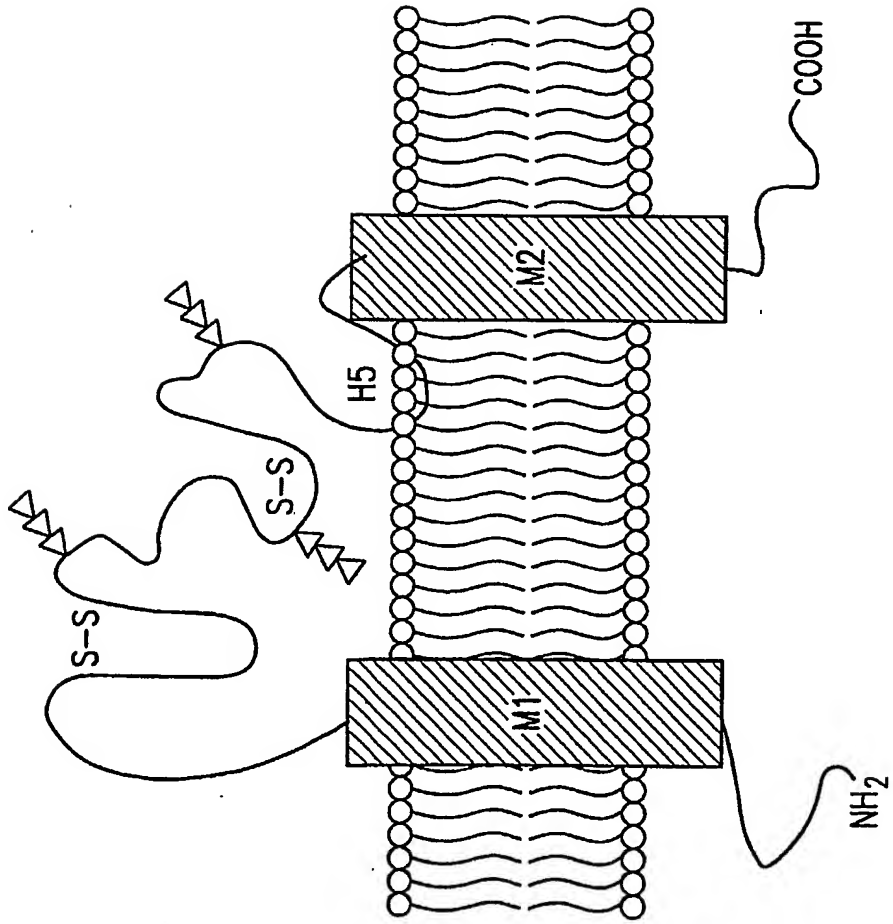


FIG.8

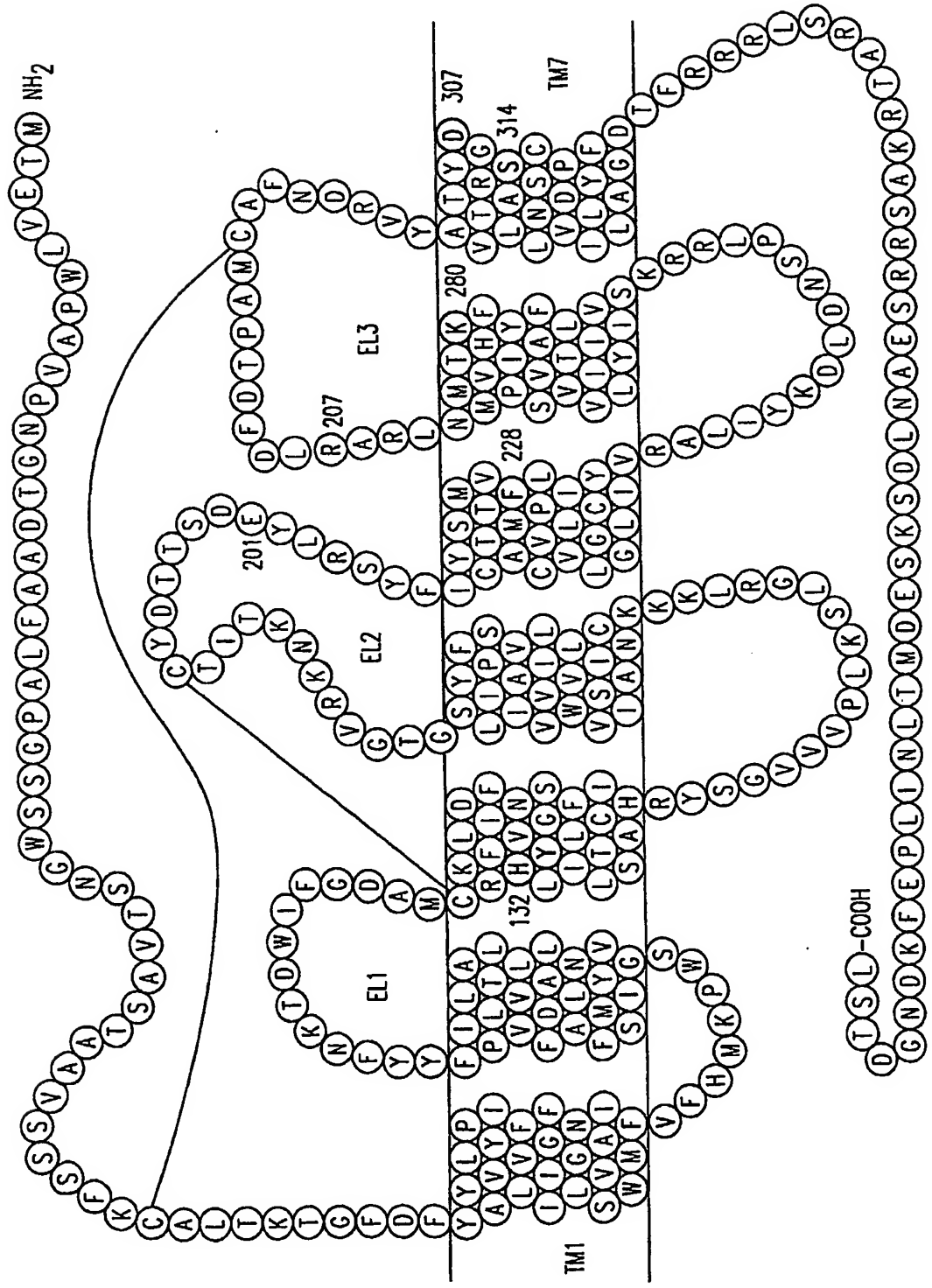
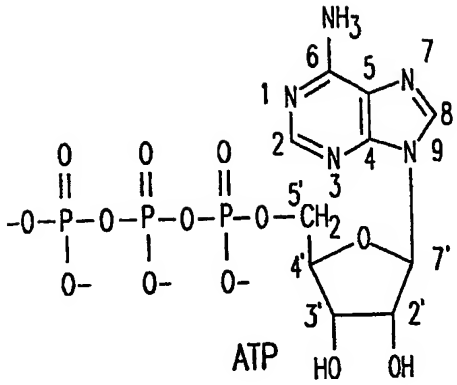


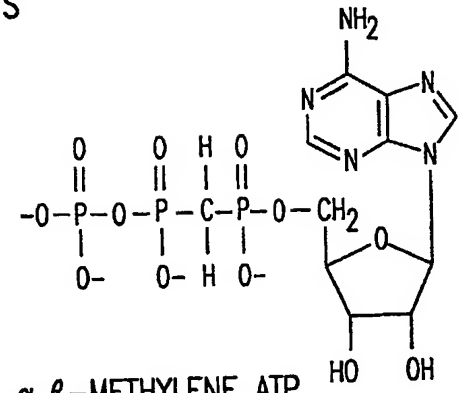
FIG.9

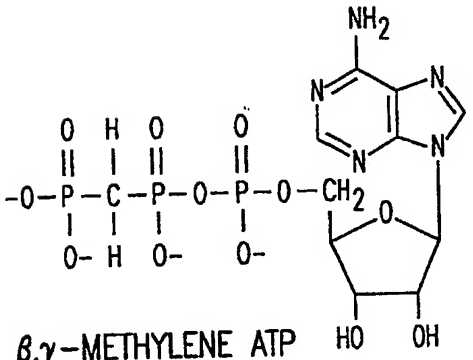
TM 3			
P2Y1 (ADP)	125	KLQRFIFHVNLYGSILFLTCISAHR	149
P2Y2 (UTP)	107	KLVRFLFYTNLYCSILFLTCISVHR	131
P2Y4 (UTP)	109	KFVRFLFYWNLYCSVLFLTCISVHR	133
P2Y6 (UDP)	100	RLVRFLFYANLAGSILFLTCISFQR	124
P2Y11 (ATP)	100	RLERFLFTCNLLCSVIFITCISLNR	124
TM 5			
P2Y1 (ADP)	215	FIYSMCTTVAMFCVFLVLILGCYGLIV	241
P2Y2 (UTP)	196	VAYSSVNLGLLFAVFFAVILVCYVLMA	222
P2Y4 (UTP)	198	VKFSSAVMGLLFGVFCLVTLVCYGLMA	224
P2Y6 (UDP)	190	MPYGMALTVICFLLFFAALLACYCLLA	215
P2Y11 (ATP)	200	RAYSLVLAGLGCGLFLLLTLAAYGALG	226
TM 6			
P2Y1 (ADP)	260	YLVIIVLTVFAVSYIPPHVMKTMNLR	285
P2Y2 (UTP)	245	RTIAVVLAVFALCFLPFHVTRTLYYS	270
P2Y4 (UTP)	245	RTIAVVLTVFAVCFVPFHITRTIYYL	270
P2Y6 (UDP)	239	RMAVVVAAAFAISFLPFHITKTAYLA	264
P2Y11 (ATP)	245	ALVASGVALYASSYVPYHIMRVINVD	270
TM7			
P2Y1 (ADP)	303	YATYQVTRGLASLNSCVDPILYPLAGDT	330
P2Y2 (UTP)	285	NMAYKVTRFLASANSCLDPVLYFLAGQR	312
P2Y4 (UTP)	285	NVVYKVTRFLASANSCLDPVLYLLTGDK	312
P2Y6 (UDP)	280	AAAYKGTRFFASANSVLDPILFYFTQKK	307
P2Y11 (ATP)	297	YVGYQVMRGLMPLASCVHPLLYMAAUPS	324

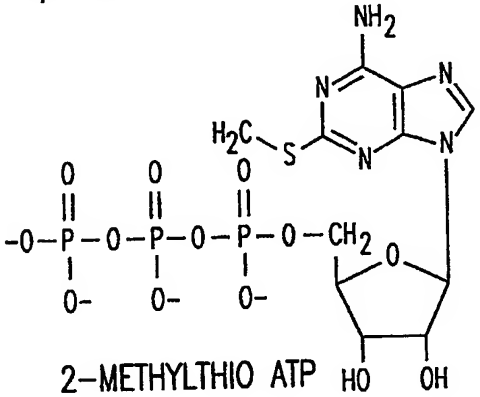
FIG.10

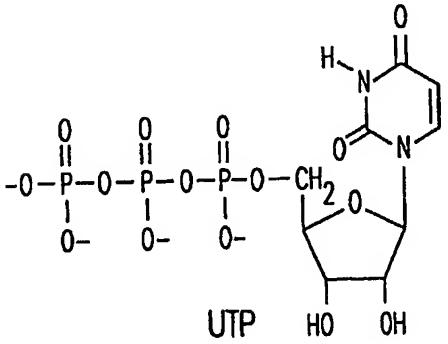
AGONISTS

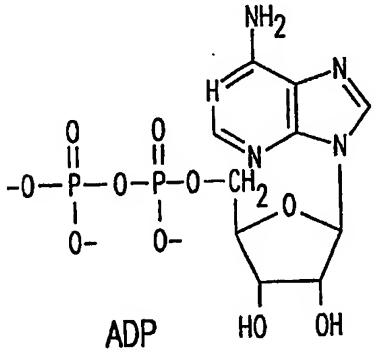

ATP

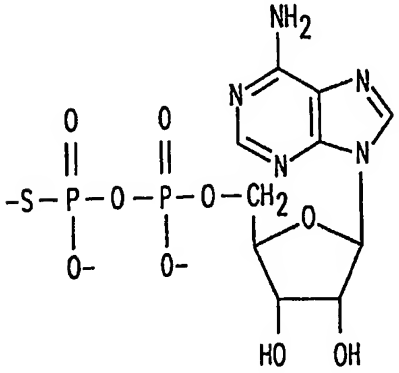

$\alpha_1\beta$-METHYLENE ATP

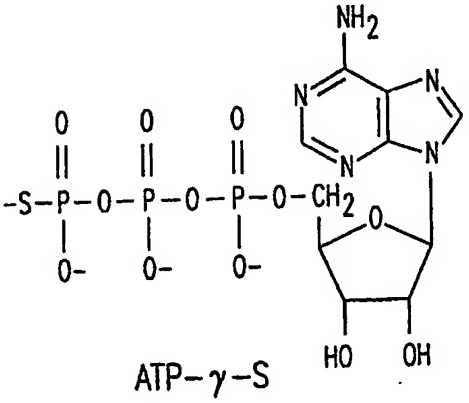

$\beta_1\gamma$-METHYLENE ATP


2-METHYLTHIO ATP


UTP


ADP


ADP-β-S


ATP-γ-S

AGONISTS (CONT'D)

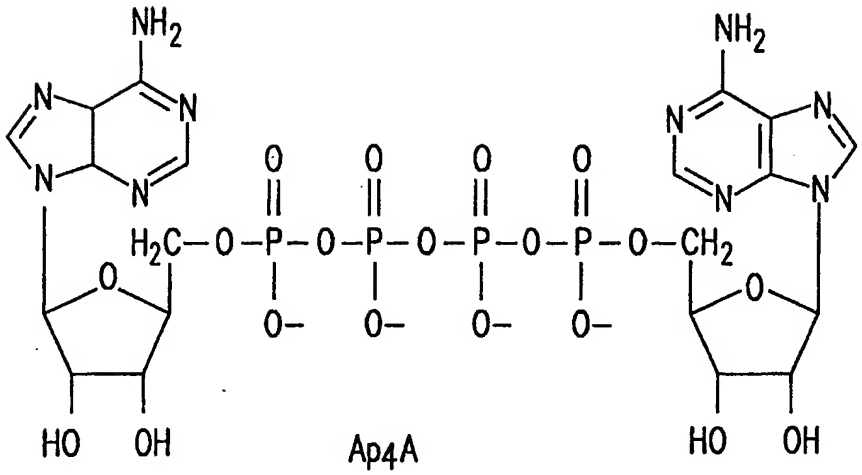


FIG.11B

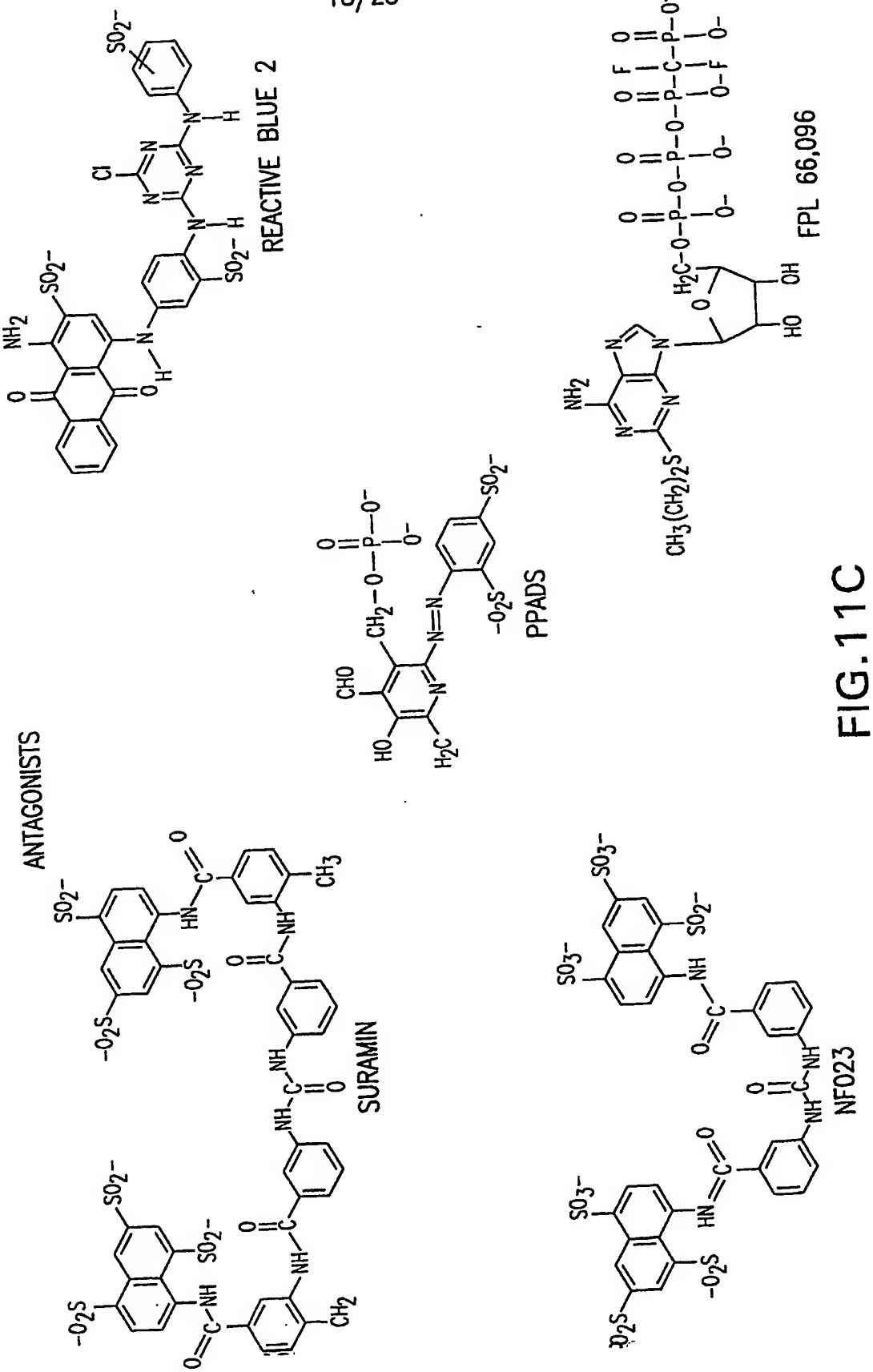


FIG.11C

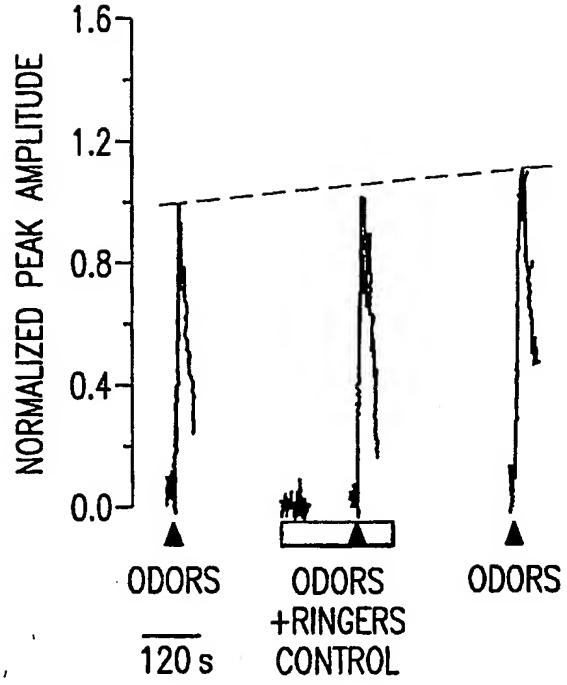


FIG.12A

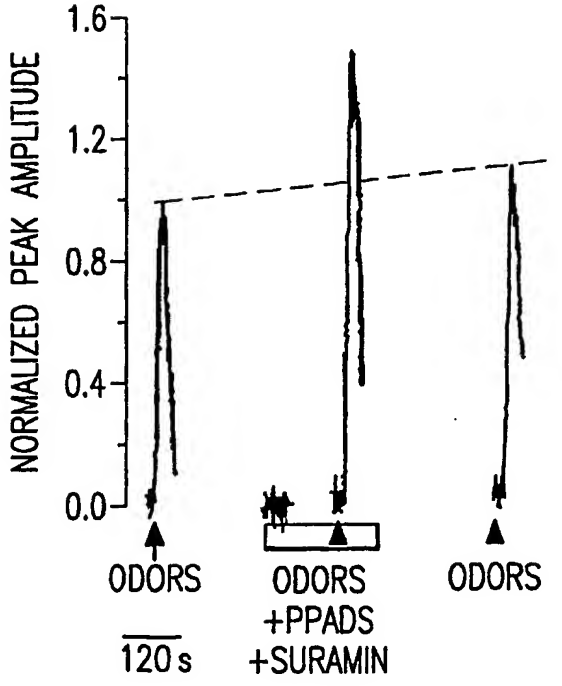


FIG.12B

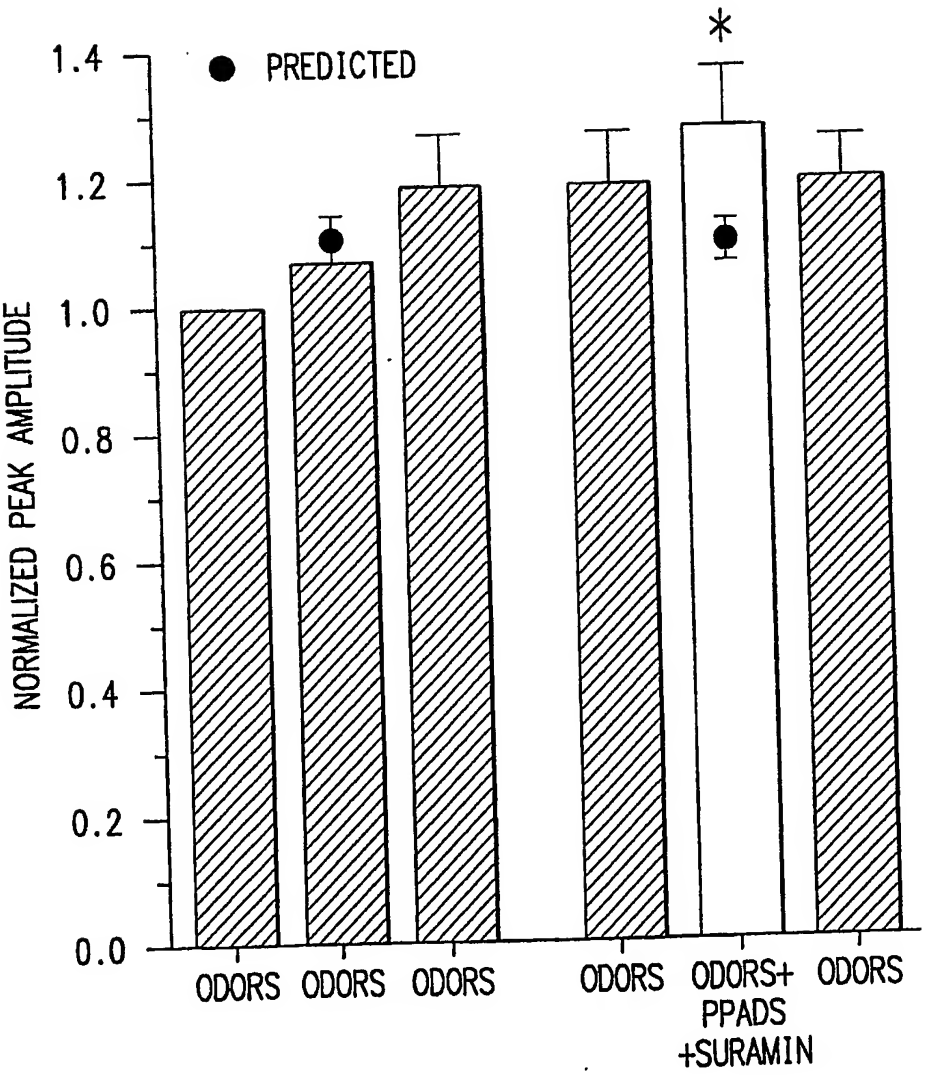

PREDICTED
*
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
NORMALIZED PEAK AMPLITUDE
ODORS
ODORS
ODORS
ODORS
ODORS+ PPADS +SURAMIN
ODORS

FIG.12C

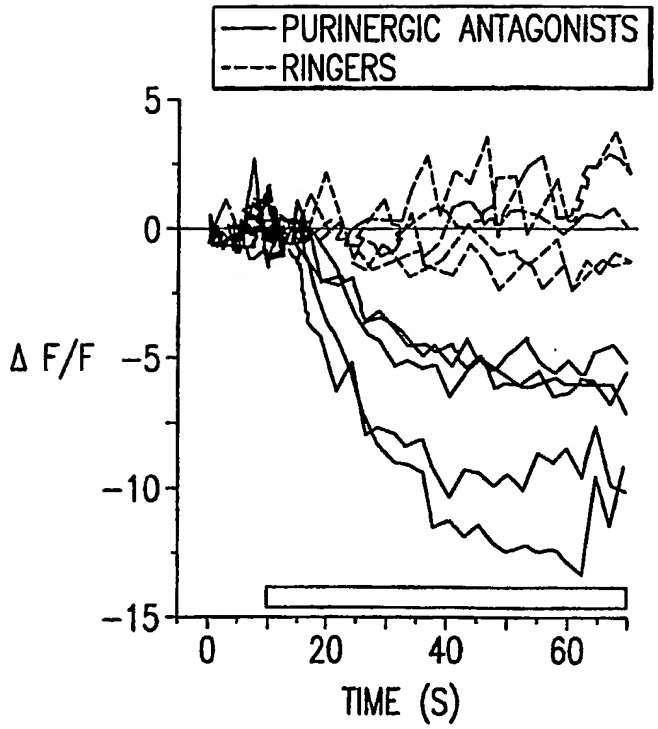

PURINERGIC ANTAGONISTS
RINGERS
5
0
−5
−10
−15
Δ F/F
0
20
40
60
TIME (S)

FIG.12D

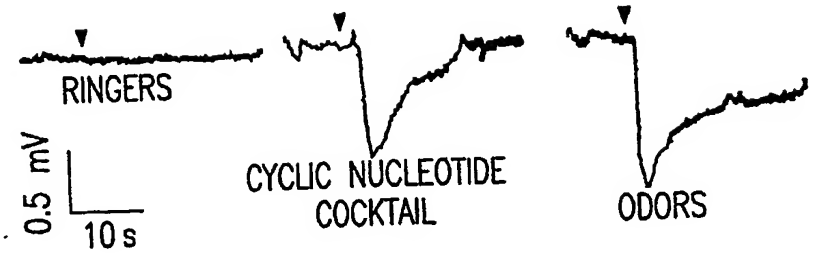


FIG.13A

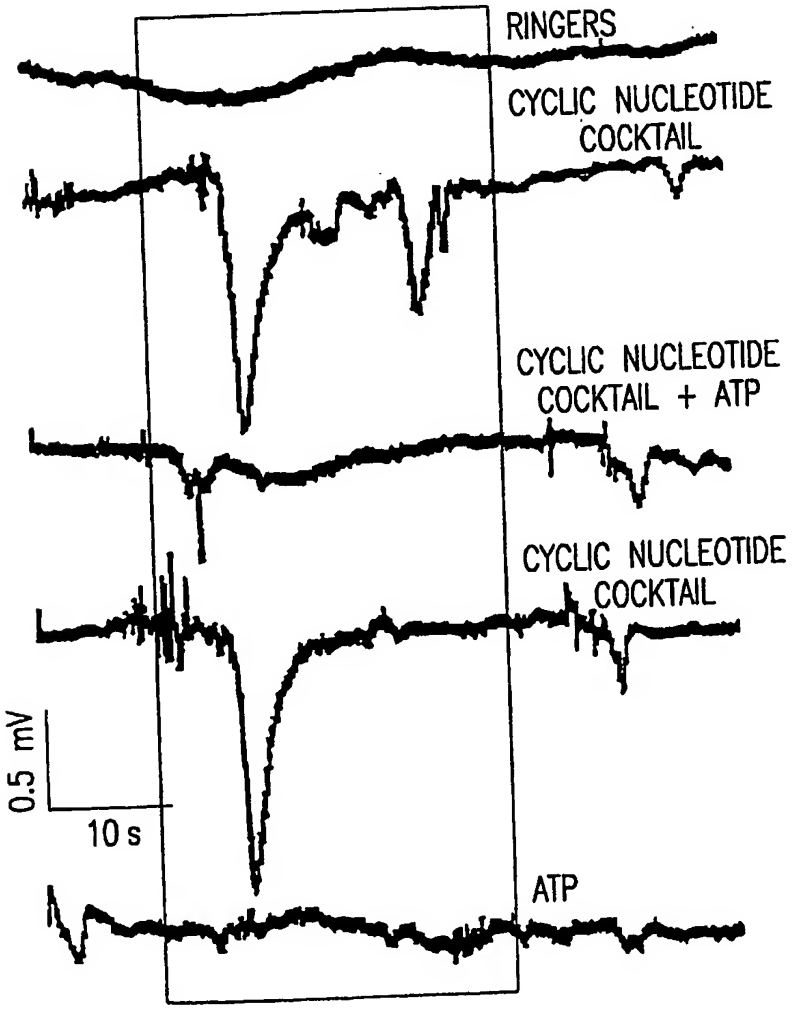


FIG.13B

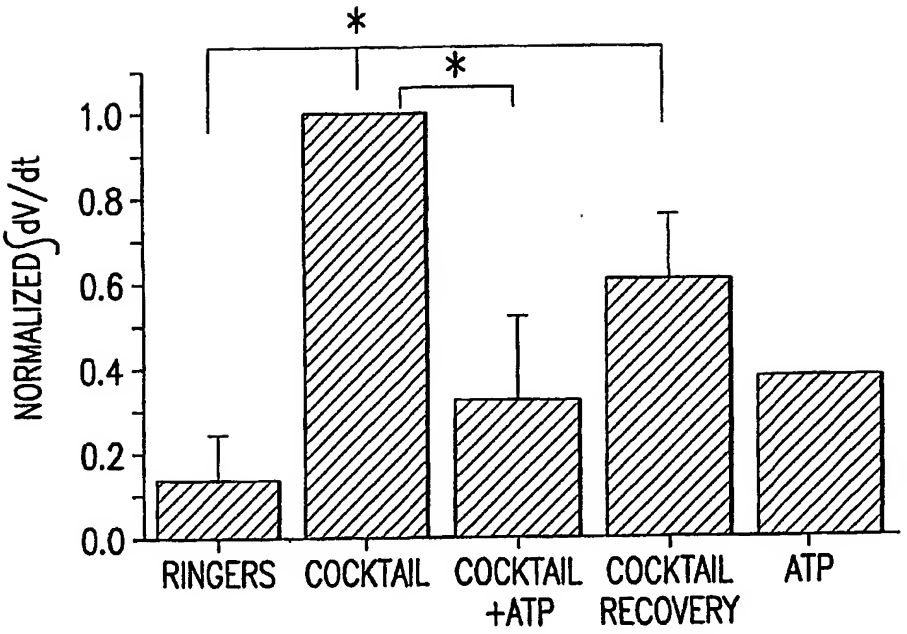

*
*
NORMALIZED ∫dV/dt
1.0
0.8
0.6
0.4
0.2
0.0
RINGERS
COCKTAIL
COCKTAIL +ATP
COCKTAIL RECOVERY
ATP

FIG.13C

SEQUENCE LISTING

<110> University of Utah Research Foundation

Lucero, Mary
Hegg, Colleen

<120> Purinergic Modulation of Smell

<130> 21101.0030P1

<150> 60/428,140
<151> 2002-11-21

<160> 26

<170> FastSEQ for Windows Version 4.0

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<211> 1452
<212> DNA
<213> Artificial Sequence

<220>
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synthetic construct

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ctacgacagg ctgggggctt ctggattata agacggagaa gtatgtgatg accaggaact 120
ggcgggtggg cgccctgcag aggctgctgc agtttgggat cgtggtctat gtggtagggt 180
gggctctcct cgccaaaaaa ggctaccagg agcgggacct ggaaccccag ttttccatca 240
tcaccaaact caaaggggtt tccgtcactc agatcaagga gcttggaaac cggctgtggg 300
atgtggccga cttcgtgaag ccacctcagg gagagaacgt gttcttcttg gtgaccaact 360
tccttgtgac gccagcccaa gttcagggca gatgcccaga gcacccgtcc gtcccactgg 420
ctaactgctg ggtcgacgaa gactgccccg aaggggaggg aggcacacac agccacggtg 480
taaaaacagg ccagtgtgtg gtgttcaatg ggacccacag gacctgtgag atctggagtt 540
ggtgcccagt ggagagtggc gttgtgccct cgaggcccct gctggcccag gcccagaact 600
tcacactgtt catcaaaaac acagtcacct tcagcaagtt caacttctct aagtccaatg 660
ccttggagac ctgggacccc acctatttta agcactgccg ctatgaacca caattcagcc 720
cctactgtcc cgtgttccgc attggggacc tcgtggccaa ggctggaggg accttcgagg 780
acctggcgtt gctgggtggc tctgtaggca tcagagttca ctgggattgt gacctggaca 840
ccggggactc tggctgctgg cctcactact ccttccagct gcaggagaag agctacaact 900
tcaggacagc cactcactgg tgggagcaac cgggtgtgga ggcccgcacc ctgctcaagc 960
tctatggaat ccgcttcgac atcctcgtca ccgggcaggc agggaagttc gggctcatcc 1020
ccacggccgt cacactgggc accggggcag cttggctggg cgtggtcacc tttttctgtg 1080
acctgctact gctgtatgtg gatagagaag cccatttcta ctggaggaca aagtatgagg 1140
aggccaaggc cccgaaagca accgccaact ctgtgtggag ggagctggcc tttgcatccc 1200
aagcccgact ggccgagtgc ctcagacgga gctcagcacc tgcacccacg gccactgctg 1260
ctgggagtca gacacagaca ccaggatggc cctgtccaag ttctgacacc cacttgccaa 1320
cccattccgg gagcctgtag ccgtttccct gctggttgag aagagagagg ggctgggcaa 1380
ggaaggaccc ctgccctgcc gagcgaaagc aaggatgagg caacagcaat gaaagaagat 1440
caagccgaat tc 1452

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<211> 399
<212> PRT
<213> Artificial Sequence

<220>
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synthetic construct

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1 5 10 15
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20 25 30
Phe Arg Leu Ile Gln Leu Val Val Leu Val Tyr Val Ile Gly Trp Val
35 40 45
Phe Leu Tyr Glu Lys Gly Tyr Gln Thr Ser Ser Gly Leu Ile Ser Ser
50 55 60
Val Ser Val Lys Leu Lys Gly Leu Ala Val Thr Gln Leu Pro Gly Leu
65 70 75 80
Gly Pro Gln Val Trp Asp Val Ala Asp Tyr Val Phe Pro Ala Gln Gly
85 90 95
Asp Asn Ser Phe Val Val Met Thr Asn Phe Ile Val Thr Pro Lys Gln
100 105 110
Thr Gln Gly Tyr Cys Ala Glu His Pro Glu Gly Gly Ile Cys Lys Glu
115 120 125
Asp Ser Gly Cys Thr Pro Gly Lys Ala Lys Arg Lys Ala Gln Gly Ile
130 135 140
Arg Thr Gly Lys Cys Val Ala Phe Asn Asp Thr Val Lys Thr Cys Glu
145 150 155 160
Ile Phe Gly Trp Cys Pro Val Glu Val Asp Asp Asp Ile Pro Arg Pro
165 170 175
Ala Leu Leu Arg Glu Ala Glu Asn Phe Thr Leu Phe Ile Lys Asn Ser
180 185 190
Ile Ser Phe Pro Arg Phe Lys Val Asn Arg Arg Asn Leu Val Glu Glu
195 200 205
Val Asn Ala Ala His Met Lys Thr Cys Leu Phe His Lys Thr Leu His
210 215 220
Pro Leu Cys Pro Val Phe Gln Leu Gly Tyr Val Val Gln Glu Ser Gly
225 230 235 240
Gln Asn Phe Ser Thr Leu Ala Glu Lys Gly Gly Val Val Gly Ile Thr
245 250 255
Ile Asp Trp His Cys Asp Leu Asp Trp His Val Arg His Cys Arg Pro
260 265 270
Ile Tyr Glu Phe His Gly Leu Tyr Glu Glu Lys Asn Leu Ser Pro Gly
275 280 285
Phe Asn Phe Arg Phe Ala Arg His Phe Val Glu Asn Gly Thr Asn Tyr
290 295 300
Arg His Leu Phe Lys Val Phe Gly Ile Arg Phe Asp Ile Leu Val Asp
305 310 315 320
Gly Lys Ala Gly Lys Phe Asp Ile Ile Pro Thr Met Thr Thr Ile Gly
325 330 335
Ser Gly Ile Gly Ile Phe Gly Val Ala Thr Val Leu Cys Asp Leu Leu
340 345 350
Leu Leu His Ile Leu Pro Lys Arg His Tyr Tyr Lys Gln Lys Lys Phe
355 360 365
Lys Tyr Ala Glu Asp Met Gly Pro Gly Ala Ala Glu Arg Asp Leu Ala
370 375 380
Ala Thr Ser Ser Thr Leu Gly Leu Gln Glu Asn Met Arg Thr Ser
385 390 395

<210> 3
<211> 1452
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:/note =
synthetic construct

<400> 3
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ctacgacagg ctgggggctt ctggattata agacggagaa gtatgtgatg accaggaact 120
ggcgggtggg cgccctgcag aggctgctgc agtttgggat cgtggtctat gtggtagggt 180
gggctctcct cgccaaaaaa ggctaccagg agcgggacct ggaaccccag ttttccatca 240
tcaccaaact caaaggggtt tccgtcactc agatcaagga gcttggaaac cggctgtggg 300
atgtggccga cttcgtgaag ccacctcagg gagagaacgt gttcttcttg gtgaccaact 360
tccttgtgac gccagcccaa gttcagggca gatgcccaga gcacccgtcc gtcccactgg 420
ctaactgctg ggtcgacgaa gactgccccg aaggggaggg aggcacacac agccacggtg 480
taaaaacagg ccagtgtgtg gtgttcaatg ggacccacag gacctgtgag atctggagtt 540
ggtgcccagt ggagagtggc gttgtgccct cgaggcccct gctggcccag gcccagaact 600
tcacactgtt catcaaaaac acagtcacct tcagcaagtt caacttctct aagtccaatg 660
ccttggagac ctgggacccc acctatttta agcactgccg ctatgaacca caattcagcc 720
cctactgtcc cgtgttccgc attggggacc tcgtggccaa ggctggaggg accttcgagg 780
acctggcgtt gctgggtggc tctgtaggca tcagagttca ctgggattgt gacctggaca 840
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ccacggccgt cacactgggc accggggcag cttggctggg cgtggtcacc ttttttctgtg 1080
acctgctact gctgtatgtg gatagagaag cccatttcta ctggaggaca aagtatgagg 1140
aggccaaggc cccgaaagca accgccaact ctgtgtggag ggagctggcc tttgcatccc 1200
aagcccgact ggccgagtgc ctcagacgga gctcagcacc tgcacccacg gccactgctg 1260
ctgggagtca gacacagaca ccaggatggc cctgtccaag ttctgacacc cacttgccaa 1320
cccattccgg gagcctgtag ccgtttccct gctggttgag aagagagagg ggctgggcaa 1380
ggaaggaccc ctgccctgcc gagcgaaagc aaggatgagg caacagcaat gaaagaagat 1440
caagccgaat tc 1452

<210> 4
<211> 472
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:/note =
synthetic construct

<400> 4
Met Val Arg Arg Leu Ala Arg Gly Cys Trp Ser Ala Phe Trp Asp Tyr
1 5 10 15
Glu Thr Pro Lys Val Ile Val Val Arg Asn Arg Arg Leu Gly Phe Val
20 25 30
His Arg Met Val Gln Leu Leu Ile Leu Leu Tyr Phe Val Trp Tyr Val
35 40 45
Phe Ile Val Gln Lys Ser Tyr Gln Asp Ser Glu Thr Gly Pro Glu Ser
50 55 60
Ser Ile Ile Thr Lys Val Lys Gly Ile Thr Met Ser Glu Asp Lys Val
65 70 75 80
Trp Asp Val Glu Glu Tyr Val Lys Pro Pro Glu Gly Gly Ser Val Val
85 90 95
Ser Ile Ile Thr Arg Ile Glu Val Thr Pro Ser Gln Thr Leu Gly Thr
100 105 110
Cys Pro Glu Ser Met Arg Val His Ser Ser Thr Cys His Ser Asp Asp
115 120 125
Asp Cys Ile Ala Gly Gln Leu Asp Met Gln Gly Asn Gly Ile Arg Thr
130 135 140

```
Gly His Cys Val Pro Tyr Tyr His Gly Asp Ser Lys Thr Cys Glu Val
145                 150                 155                 160
Ser Ala Trp Cys Pro Val Glu Asp Gly Thr Ser Asp Asn His Phe Leu
                165                 170                 175
Gly Lys Met Ala Pro Asn Phe Thr Ile Leu Ile Lys Asn Ser Ile His
            180                 185                 190
Tyr Pro Lys Phe Lys Phe Ser Lys Gly Asn Ile Ala Ser Gln Lys Ser
        195                 200                 205
Asp Tyr Leu Lys His Cys Thr Phe Asp Gln Asp Ser Asp Pro Tyr Cys
    210                 215                 220
Pro Ile Phe Arg Leu Gly Phe Ile Val Glu Lys Ala Gly Glu Asn Phe
225                 230                 235                 240
Thr Glu Leu Ala His Lys Gly Gly Val Ile Gly Val Ile Ile Asn Trp
                245                 250                 255
Asn Cys Asp Leu Asp Leu Ser Glu Ser Glu Cys Asn Pro Lys Tyr Ser
            260                 265                 270
Phe Arg Arg Leu Asp Pro Lys Tyr Asp Pro Ala Ser Ser Gly Tyr Asn
        275                 280                 285
Phe Arg Phe Ala Lys Tyr Tyr Lys Ile Asn Gly Thr Thr Thr Thr Arg
    290                 295                 300
Thr Leu Ile Lys Ala Tyr Gly Ile Arg Ile Asp Val Ile Val His Gly
305                 310                 315                 320
Gln Ala Gly Lys Phe Ser Leu Ile Pro Thr Ile Ile Asn Leu Ala Thr
                325                 330                 335
Ala Leu Thr Ser Ile Gly Val Gly Ser Phe Leu Cys Asp Trp Ile Leu
            340                 345                 350
Leu Thr Phe Met Asn Lys Asn Lys Leu Tyr Ser His Lys Lys Phe Asp
        355                 360                 365
Lys Val Arg Thr Pro Lys His Pro Ser Ser Arg Trp Pro Val Thr Leu
    370                 375                 380
Ala Leu Val Leu Gly Gln Ile Pro Pro Pro Pro Ser His Tyr Ser Gln
385                 390                 395                 400
Asp Gln Pro Pro Ser Pro Pro Ser Gly Glu Gly Pro Thr Leu Gly Glu
                405                 410                 415
Gly Ala Glu Leu Pro Leu Ala Val Gln Ser Pro Arg Pro Cys Ser Ile
            420                 425                 430
Ser Ala Leu Thr Glu Gln Val Val Asp Thr Leu Gly Gln His Met Gly
        435                 440                 445
Gln Arg Pro Pro Val Pro Glu Pro Ser Gln Gln Asp Ser Thr Ser Thr
    450                 455                 460
Asp Pro Lys Gly Leu Ala Gln Leu
465                 470
```

<210> 5
<211> 1452
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:/note =
synthetic construct

<400> 5
gaattcggct gatcccgcgg caggtgctag caggagctgg cagcatgggc tccccagggg 60
ctacgacagg ctgggggctt ctggattata agacggagaa gtatgtgatg accaggaact 120
ggcgggtggg cgccctgcag aggctgctgc agtttgggat cgtggtctat gtggtagggt 180
gggctctcct cgccaaaaaa ggctaccagg agcgggacct ggaaccccag ttttccatca 240
tcaccaaact caaaggggtt tccgtcactc agatcaagga gcttggaaac cggctgtggg 300
atgtggccga cttcgtgaag ccacctcagg gagagaacgt gttcttcttg gtgaccaact 360
tccttgtgac gccagcccaa gttcagggca gatgcccaga gcacccgtcc gtcccactgg 420

ctaactgctg ggtcgacgaa gactgccccg aaggggaggg aggcacacac agccacggtg 480
taaaaacagg ccagtgtgtg gtgttcaatg ggacccacag gacctgtgag atctggagtt 540
ggtgcccagt ggagagtggc gttgtgccct cgaggcccct gctggcccag gcccagaact 600
tcacactgtt catcaaaaac acagtcacct tcagcaagtt caacttctct aagtccaatg 660
ccttggagac ctgggacccc acctatttta agcactgccg ctatgaacca caattcagcc 720
cctactgtcc cgtgttccgc attggggacc tcgtggccaa ggctggaggg accttcgagg 780
acctggcgtt gctgggtggc tctgtaggca tcagagttca ctgggattgt gacctggaca 840
ccggggactc tggctgctgg cctcactact ccttccagct gcaggagaag agctacaact 900
tcaggacagc cactcactgg tgggagcaac cgggtgtgga ggcccgcacc ctgctcaagc 960
tctatggaat ccgcttcgac atcctcgtca ccgggcaggc agggaagttc gggctcatcc 1020
ccacggccgt cacactgggc accggggcag cttggctggg cgtggtcacc tttttctgtg 1080
acctgctact gctgtatgtg gatagagaag cccatttcta ctggaggaca aagtatgagg 1140
aggccaaggc cccgaaagca accgccaact ctgtgtggag ggagctggcc tttgcatccc 1200
aagcccgact ggccgagtgc ctcagacgga gctcagcacc tgcacccacg gccactgctg 1260
ctgggagtca gacacagaca ccaggatggc cctgtccaag ttctgacacc cacttgccaa 1320
cccattccgg gagcctgtag ccgtttccct gctggttgag aagagagagg ggctgggcaa 1380
ggaaggaccc ctgccctgcc gagcgaaagc aaggatgagg caacagcaat gaaagaagat 1440
caagccgaat tc 1452

<210> 6
<211> 397
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:/note =
synthetic construct

<400> 6
Met Asn Cys Ile Ser Asp Phe Phe Thr Tyr Glu Thr Thr Lys Ser Val
1 5 10 15
Val Val Lys Ser Trp Thr Ile Gly Ile Ile Asn Arg Val Val Gln Leu
20 25 30
Leu Ile Ile Ser Tyr Phe Val Gly Trp Val Phe Leu His Glu Lys Ala
35 40 45
Tyr Gln Val Arg Asp Thr Ala Ile Glu Ser Ser Val Val Thr Lys Val
50 55 60
Lys Gly Ser Gly Leu Tyr Ala Asn Arg Val Met Asp Val Ser Asp Tyr
65 70 75 80
Val Thr Pro Pro Gln Gly Thr Ser Val Phe Val Ile Ile Thr Lys Met
85 90 95
Ile Val Thr Glu Asn Gln Met Gln Gly Phe Cys Pro Glu Ser Glu Glu
100 105 110
Lys Tyr Arg Cys Val Ser Asp Ser Gln Cys Gly Pro Glu Pro Leu Pro
115 120 125
Gly Gly Gly Ile Leu Thr Gly Arg Cys Val Asn Tyr Ser Ser Val Leu
130 135 140
Arg Thr Cys Glu Ile Gln Gly Trp Cys Pro Thr Glu Val Asp Thr Val
145 150 155 160
Glu Thr Pro Ile Met Met Glu Ala Glu Asn Phe Thr Ile Phe Ile Lys
165 170 175
Asn Ser Ile Arg Phe Pro Leu Phe Asn Phe Glu Lys Gly Asn Leu Leu
180 185 190
Pro Asn Leu Thr Ala Arg Asp Met Lys Thr Cys Arg Phe His Pro Asp
195 200 205
Lys Asp Pro Phe Cys Pro Ile Leu Arg Val Gly Asp Val Val Lys Phe
210 215 220
Ala Gly Gln Asp Phe Ala Lys Leu Ala Arg Thr Gly Gly Val Leu Gly
225 230 235 240
Ile Lys Ile Gly Trp Val Cys Asp Leu Asp Lys Ala Trp Asp Gln Cys
245 250 255

```
Ile Pro Lys Tyr Ser Phe Thr Arg Leu Asp Ser Val Ser Glu Lys Ser
            260                 265                 270
Ser Val Ser Pro Gly Tyr Asn Phe Arg Phe Ala Lys Tyr Tyr Lys Met
        275                 280                 285
Glu Asn Gly Ser Glu Tyr Arg Thr Leu Leu Lys Ala Phe Gly Ile Arg
    290                 295                 300
Phe Asp Val Leu Val Tyr Gly Asn Ala Gly Lys Phe Asn Ile Ile Pro
305                 310                 315                 320
Thr Ile Ile Ser Ser Val Ala Ala Phe Thr Ser Val Gly Val Gly Thr
                325                 330                 335
Val Leu Cys Asp Ile Ile Leu Leu Asn Phe Leu Lys Gly Ala Asp Gln
            340                 345                 350
Tyr Lys Ala Lys Lys Phe Glu Glu Val Asn Glu Thr Thr Leu Lys Ile
        355                 360                 365
Ala Ala Leu Thr Asn Pro Val Tyr Pro Ser Asp Gln Thr Thr Ala Glu
    370                 375                 380
Lys Gln Ser Thr Asp Ser Gly Ala Phe Ser Ile Gly His
385                 390                 395
```

<210> 7
<211> 1452
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:/note =
synthetic construct

<400> 7

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gaattcggct gatcccgcgg caggtgctag caggagctgg cagcatgggc tccccagggg 60
ctacgacagg ctgggggctt ctggattata agacggagaa gtatgtgatg accaggaact 120
ggcgggtggg cgccctgcag aggctgctgc agtttgggat cgtggtctat gtggtagggt 180
gggctctcct cgccaaaaaa ggctaccagg agcgggacct ggaaccccag ttttccatca 240
tcaccaaact caaaggggtt tccgtcactc agatcaagga gcttggaaac cggctgtggg 300
atgtggccga cttcgtgaag ccacctcagg gagagaacgt gttcttcttg gtgaccaact 360
tccttgtgac gccagcccaa gttcagggca gatgcccaga gcacccgtcc gtcccactgg 420
ctaactgctg ggtcgacgaa gactgccccg aaggggaggg aggcacacac agccacggtg 480
taaaaacagg ccagtgtgtg gtgttcaatg ggacccacag gacctgtgag atctggagtt 540
ggtgcccagt ggagagtggc gttgtgccct cgaggcccct gctggcccag gcccagaact 600
tcacactgtt catcaaaaac acagtcacct tcagcaagtt caacttctct aagtccaatg 660
ccttggagac ctgggacccc acctatttta agcactgccg ctatgaacca caattcagcc 720
cctactgtcc cgtgttccgc attggggacc tcgtggccaa ggctggaggg accttcgagg 780
acctggcgtt gctgggtggc tctgtaggca tcagagttca ctgggattgt gacctggaca 840
ccgggactc tggctgctgg cctcactact ccttccagct gcaggagaag agctacaact 900
tcaggacagc cactcactgg tgggagcaac cgggtgtgga ggcccgcacc ctgctcaagc 960
tctatggaat ccgcttcgac atcctcgtca ccgggcaggc agggaagttc gggctcatcc 1020
ccacggccgt cacactgggc accggggcag cttggctggg cgtggtcacc tttttctgtg 1080
acctgctact gctgtatgtg gatagagaag cccatttcta ctggaggaca aagtatgagg 1140
aggccaaggc cccgaaagca accgccaact ctgtgtggag ggagctggcc tttgcatccc 1200
aagcccgact ggccgagtgc ctcagacgga gctcagcacc tgcacccacg gccactgctg 1260
ctgggagtca gacacagaca ccaggatggc cctgtccaag ttctgacacc cacttgccaa 1320
cccattccgg gagcctgtag ccgtttccct gctggttgag aagagagagg ggctgggcaa 1380
ggaaggaccc ctgccctgcc gagcgaaagc aaggatgagg caacagcaat gaaagaagat 1440
caagccgaat tc                                                    1452
```

<210> 8
<211> 388
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:/note =
synthetic construct

<400> 8
Met Ala Gly Cys Cys Ser Ala Leu Ala Ala Phe Leu Phe Glu Tyr Asp
1 5 10 15
Thr Pro Arg Ile Val Leu Ile Arg Ser Arg Lys Val Gly Leu Met Asn
20 25 30
Arg Ala Val Gln Leu Leu Ile Leu Ala Tyr Val Ile Gly Trp Val Phe
35 40 45
Val Trp Glu Lys Gly Tyr Gln Glu Thr Asp Ser Val Val Ser Ser Val
50 55 60
Thr Thr Lys Val Lys Gly Val Ala Val Thr Asn Thr Ser Lys Leu Gly
65 70 75 80
Phe Arg Ile Trp Asp Val Ala Asp Tyr Val Ile Pro Ala Gln Glu Glu
85 90 95
Asn Ser Leu Phe Val Met Thr Asn Val Ile Leu Thr Met Asn Gln Thr
100 105 110
Gln Gly Leu Cys Pro Glu Ile Pro Asp Ala Thr Thr Val Cys Lys Ser
115 120 125
Asp Ala Ser Cys Thr Ala Gly Ser Ala Gly Thr His Ser Asn Gly Val
130 135 140
Ser Thr Gly Arg Cys Val Ala Phe Asn Gly Ser Val Lys Thr Cys Glu
145 150 155 160
Val Ala Ala Trp Cys Pro Val Glu Asp Asp Thr His Val Pro Gln Pro
165 170 175
Ala Phe Leu Lys Ala Ala Glu Asn Phe Thr Leu Leu Val Lys Asn Asn
180 185 190
Ile Trp Tyr Pro Lys Phe Asn Phe Ser Lys Arg Asn Ile Leu Pro Asn
195 200 205
Ile Thr Thr Thr Tyr Leu Lys Ser Cys Ile Tyr Asp Ala Lys Thr Asp
210 215 220
Pro Phe Cys Pro Ile Phe Arg Leu Gly Lys Ile Val Glu Asn Ala Gly
225 230 235 240
His Ser Phe Gln Asp Met Ala Val Glu Gly Gly Ile Met Gly Ile Gln
245 250 255
Val Asn Trp Asp Cys Asn Leu Asp Arg Ala Ala Ser Leu Cys Leu Pro
260 265 270
Arg Tyr Ser Phe Arg Arg Leu Asp Thr Arg Asp Val Glu His Asn Val
275 280 285
Ser Pro Gly Tyr Asn Phe Arg Phe Ala Lys Tyr Tyr Arg Asp Leu Ala
290 295 300
Gly Asn Glu Gln Arg Thr Leu Ile Lys Ala Tyr Gly Ile Arg Phe Asp
305 310 315 320
Ile Ile Val Phe Gly Lys Ala Gly Lys Phe Asp Ile Ile Pro Thr Met
325 330 335
Ile Asn Ile Gly Ser Gly Leu Ala Leu Leu Gly Met Ala Thr Val Leu
340 345 350
Cys Asp Ile Ile Val Leu Tyr Cys Met Lys Lys Arg Leu Tyr Tyr Arg
355 360 365
Glu Lys Lys Tyr Lys Tyr Val Glu Asp Tyr Glu Gln Gly Leu Ala Ser
370 375 380
Glu Leu Asp Gln
385

<210> 9
<211> 1978
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:/note =
synthetic construct

<400> 9
ggcacgaggg tccgcaagcc cggctgagag cgcgccatgg ggcaggcggg ctgcaagggg 60
ctctgcctgt cgctgttcga ctacaagacc gagaagtatg tcatcgccaa gaacaagaag 120
gtgggcctgc tgtaccggct gctgcaggcc tccatcctgg cgtacctggt cgtatgggtg 180
ttcctgataa agaagggtta ccaagacgtc gacacctccc tgcagagtgc tgtcatcacc 240
aaagtcaagg gcgtggcctt caccaacacc tcggatcttg ggcagcggat ctgggatgtc 300
gccgactacg tcattccagc ccagggagag aacgtctttt ttgtggtcac caacctgatt 360
gtgaccccca accagcggca gaacgtctgt gctgagaatg aaggcattcc tgatggcgcg 420
tgctccaagg acagcgactg ccacgctggg gaagcggtta cagctggaaa cggagtgaag 480
accggccgct gcctgcggag agggaacttg gccaggggca cctgtgagat ctttgcctgg 540
tgcccgttgg agacaagctc caggccggag gagccattcc tgaaggaggc cgaagacttc 600
accattttca taaagaacca catccgtttc cccaaattca acttctccaa aaacaatgtg 660
atggacgtca aggacagatc tttcctgaaa tcatgccact ttggccccaa gaaccactac 720
tgccccatct tccgactggg ctccatcgtc cgctgggccg ggagcgactt ccaggatata 780
gccctgcgag gtggcgtgat aggaattaat attgaatgga actgtgatct tgataaagct 840
gcctctgagt gccaccctca ctattctttt agccgtctgg acaataaact ttcaaagtct 900
gtctcctccg ggtacaactt cagatttgcc agatattacc gagacgcagc cggggtggag 960
ttccgcaccc tgatgaaagc ctacgggatc cgctttgacg tgatggtgaa cggcaagggt 1020
gctttcttct gcgacctggt actcatctac ctcatcaaaa agagagagtt ttaccgtgac 1080
aagaagtacg aggaagtgag gggcctagaa gacagttccc aggaggccga ggacgaggca 1140
tcggggctgg ggctatctga gcagctcaca tctgggccag ggctgctggg gatgccggag 1200
cagcaggagc tgcaggagcc acccgaggcg aagcgtggaa gcagcagtca gaaggggaac 1260
ggatctgtgt gcccacagct cctggagccc cacaggagca cgtgaattgc ctctgcttac 1320
gttcaggccc tgtcctaaac ccagccgtct agcacccagt gatcccatgc ctttgggaat 1380
cccaggatgc tgcccaacgg gaaatttgta cattgggtgc tatcaatgcc acatcacagg 1440
gaccagccat cacagagcaa agtgacctcc acgtctgatg ctggggtcat caggacggac 1500
ccatcatggc tgtcttttg ccccaccccc tgccgtcagt tcttcctttc tccgtggctg 1560
gcttcccgca ctagggaacg ggttgtaaat ggggaacatg acttccttcc ggagtccttg 1620
agcacctcag ctaaggaccg cagtgccctg tagagttcct agattacctc actgggaata 1680
gcattgtgcg tgtccggaaa agggctccat ttggttccag cccactcccc tctgcaagtg 1740
ccacagcttc cctcagagca tactctccag tggatccaag tactctctct cctaaagaca 1800
ccaccttcct gccagctgtt tgcccttagg ccagtacaca gaattaaagt gggggagatg 1860
gcagacgctt tctgggacct gcccaagata tgtattctct gacactctta tttggtcata 1920
aaacaataaa tggtgtcaat ttcaaaaaaa aaaaaaaaaa aaaaaaaaaa aaaaaaaa 1978

<210> 10
<211> 422
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:/note =
synthetic construct

<400> 10
Met Gly Gln Ala Gly Cys Lys Gly Leu Cys Leu Ser Leu Phe Asp Tyr
1 5 10 15
Lys Thr Glu Lys Tyr Val Ile Ala Lys Asn Lys Lys Val Gly Leu Leu
20 25 30
Tyr Arg Leu Leu Gln Ala Ser Ile Leu Ala Tyr Leu Val Val Trp Val
35 40 45
Phe Leu Ile Lys Lys Gly Tyr Gln Asp Val Asp Thr Ser Leu Gln Ser
50 55 60
Ala Val Ile Thr Lys Val Lys Gly Val Ala Phe Thr Asn Thr Ser Asp
65 70 75 80

```
Leu Gly Gln Arg Ile Trp Asp Val Ala Asp Tyr Val Ile Pro Ala Gln
                85                  90                  95
Gly Glu Asn Val Phe Phe Val Val Thr Asn Leu Ile Val Thr Pro Asn
            100                 105                 110
Gln Arg Gln Asn Val Cys Ala Glu Asn Glu Gly Ile Pro Asp Gly Ala
        115                 120                 125
Cys Ser Lys Asp Ser Asp Cys His Ala Gly Glu Ala Val Thr Ala Gly
    130                 135                 140
Asn Gly Val Lys Thr Gly Arg Cys Leu Arg Arg Gly Asn Leu Ala Arg
145                 150                 155                 160
Gly Thr Cys Glu Ile Phe Ala Trp Cys Pro Leu Glu Thr Ser Ser Arg
                165                 170                 175
Pro Glu Glu Pro Phe Leu Lys Glu Ala Glu Asp Phe Thr Ile Phe Ile
            180                 185                 190
Lys Asn His Ile Arg Phe Pro Lys Phe Asn Phe Ser Lys Asn Asn Val
        195                 200                 205
Met Asp Val Lys Asp Arg Ser Phe Leu Lys Ser Cys His Phe Gly Pro
    210                 215                 220
Lys Asn His Tyr Cys Pro Ile Phe Arg Leu Gly Ser Ile Val Arg Trp
225                 230                 235                 240
Ala Gly Ser Asp Phe Gln Asp Ile Ala Leu Arg Gly Gly Val Ile Gly
                245                 250                 255
Ile Asn Ile Glu Trp Asn Cys Asp Leu Asp Lys Ala Ala Ser Glu Cys
            260                 265                 270
His Pro His Tyr Ser Phe Ser Arg Leu Asp Asn Lys Leu Ser Lys Ser
        275                 280                 285
Val Ser Ser Gly Tyr Asn Phe Arg Phe Ala Arg Tyr Tyr Arg Asp Ala
    290                 295                 300
Ala Gly Val Glu Phe Arg Thr Leu Met Lys Ala Tyr Gly Ile Arg Phe
305                 310                 315                 320
Asp Val Met Val Asn Gly Lys Gly Ala Phe Phe Cys Asp Leu Val Leu
                325                 330                 335
Ile Tyr Leu Ile Lys Lys Arg Glu Phe Tyr Arg Asp Lys Lys Tyr Glu
            340                 345                 350
Glu Val Arg Gly Leu Glu Asp Ser Ser Gln Glu Ala Glu Asp Glu Ala
        355                 360                 365
Ser Gly Leu Gly Leu Ser Glu Gln Leu Thr Ser Gly Pro Gly Leu Leu
    370                 375                 380
Gly Met Pro Glu Gln Gln Glu Leu Gln Glu Pro Pro Glu Ala Lys Arg
385                 390                 395                 400
Gly Ser Ser Ser Gln Lys Gly Asn Gly Ser Val Cys Pro Gln Leu Leu
                405                 410                 415
Glu Pro His Arg Ser Thr
            420
```

<210> 11
<211> 1452
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:/note =
synthetic construct

<400> 11
gaattcggct gatcccgcgg caggtgctag caggagctgg cagcatgggc tccccagggg 60
ctacgacagg ctgggggctt ctggattata agacggagaa gtatgtgatg accaggaact 120
ggcgggtggg cgccctgcag aggctgctgc agtttgggat cgtggtctat gtggtagggt 180
gggctctcct cgccaaaaaa ggctaccagg agcgggacct ggaacccccag ttttccatca 240
tcaccaaact caaaggggtt tccgtcactc agatcaagga gcttggaaac cggctgtggg 300

atgtggccga cttcgtgaag ccacctcagg gagagaacgt gttcttcttg gtgaccaact 360
tccttgtgac gccagcccaa gttcagggca gatgcccaga gcacccgtcc gtcccactgg 420
ctaactgctg ggtcgacgaa gactgccccg aaggggaggg aggcacacac agccacggtg 480
taaaaacagg ccagtgtgtg gtgttcaatg ggacccacag gacctgtgag atctggagtt 540
ggtgcccagt ggagagtggc gttgtgccct cgaggcccct gctggcccag gcccagaact 600
tcacactgtt catcaaaaac acagtcacct tcagcaagtt caacttctct aagtccaatg 660
ccttggagac ctgggacccc acctatttta agcactgccg ctatgaacca caattcagcc 720
cctactgtcc cgtgttccgc attggggacc tcgtggccaa ggctggaggg accttcgagg 780
acctggcgtt gctgggtggc tctgtaggca tcagagttca ctgggattgt gacctggaca 840
ccggggactc tggctgctgg cctcactact ccttccagct gcaggagaag agctacaact 900
tcaggacagc cactcactgg tgggagcaac cgggtgtgga ggcccgcacc ctgctcaagc 960
tctatggaat ccgcttcgac atcctcgtca ccgggcaggc agggaagttc gggctcatcc 1020
ccacggccgt cacactgggc accggggcag cttggctggg cgtggtcacc tttttctgtg 1080
acctgctact gctgtatgtg gatagagaag cccatttcta ctggaggaca aagtatgagg 1140
aggccaaggc cccgaaagca accgccaact ctgtgtggag ggagctggcc tttgcatccc 1200
aagcccgact ggccgagtgc ctcagacgga gctcagcacc tgcacccacg gccactgctg 1260
ctgggagtca gacacagaca ccaggatggc cctgtccaag ttctgacacc cacttgccaa 1320
cccattccgg gagcctgtag ccgtttccct gctggttgag aagagagagg ggctgggcaa 1380
ggaaggaccc ctgccctgcc gagcgaaagc aaggatgagg caacagcaat gaaagaagat 1440
caagccgaat tc 1452

<210> 12
<211> 431
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:/note =
synthetic construct

<400> 12
Met Gly Ser Pro Gly Ala Thr Thr Gly Trp Gly Leu Leu Asp Tyr Lys
1 5 10 15
Thr Glu Lys Tyr Val Met Thr Arg Asn Trp Arg Val Gly Ala Leu Gln
20 25 30
Arg Leu Leu Gln Phe Gly Ile Val Val Tyr Val Val Gly Trp Ala Leu
35 40 45
Leu Ala Lys Lys Gly Tyr Gln Glu Arg Asp Leu Glu Pro Gln Phe Ser
50 55 60
Ile Ile Thr Lys Leu Lys Gly Val Ser Val Thr Gln Ile Lys Glu Leu
65 70 75 80
Gly Asn Arg Leu Trp Asp Val Ala Asp Phe Val Lys Pro Pro Gln Gly
85 90 95
Glu Asn Val Phe Phe Leu Val Thr Asn Phe Leu Val Thr Pro Ala Gln
100 105 110
Val Gln Gly Arg Cys Pro Glu His Pro Ser Val Pro Leu Ala Asn Cys
115 120 125
Trp Val Asp Glu Asp Cys Pro Glu Gly Glu Gly Gly Thr His Ser His
130 135 140
Gly Val Lys Thr Gly Gln Cys Val Val Phe Asn Gly Thr His Arg Thr
145 150 155 160
Cys Glu Ile Trp Ser Trp Cys Pro Val Glu Ser Gly Val Val Pro Ser
165 170 175
Arg Pro Leu Leu Ala Gln Ala Gln Asn Phe Thr Leu Phe Ile Lys Asn
180 185 190
Thr Val Thr Phe Ser Lys Phe Asn Phe Ser Lys Ser Asn Ala Leu Glu
195 200 205
Thr Trp Asp Pro Thr Tyr Phe Lys His Cys Arg Tyr Glu Pro Gln Phe
210 215 220
Ser Pro Tyr Cys Pro Val Phe Arg Ile Gly Asp Leu Val Ala Lys Ala
225 230 235 240

```
Gly Gly Thr Phe Glu Asp Leu Ala Leu Leu Gly Gly Ser Val Gly Ile
                245                 250                 255
Arg Val His Trp Asp Cys Asp Leu Asp Thr Gly Asp Ser Gly Cys Trp
            260                 265                 270
Pro His Tyr Ser Phe Gln Leu Gln Glu Lys Ser Tyr Asn Phe Arg Thr
        275                 280                 285
Ala Thr His Trp Trp Glu Gln Pro Gly Val Glu Ala Arg Thr Leu Leu
    290                 295                 300
Lys Leu Tyr Gly Ile Arg Phe Asp Ile Leu Val Thr Gly Gln Ala Gly
305                 310                 315                 320
Lys Phe Gly Leu Ile Pro Thr Ala Val Thr Leu Gly Thr Gly Ala Ala
                325                 330                 335
Trp Leu Gly Val Val Thr Phe Phe Cys Asp Leu Leu Leu Leu Tyr Val
            340                 345                 350
Asp Arg Glu Ala His Phe Tyr Trp Arg Thr Lys Tyr Glu Glu Ala Lys
        355                 360                 365
Ala Pro Lys Ala Thr Ala Asn Ser Val Trp Arg Glu Leu Ala Phe Ala
    370                 375                 380
Ser Gln Ala Arg Leu Ala Glu Cys Leu Arg Arg Ser Ser Ala Pro Ala
385                 390                 395                 400
Pro Thr Ala Thr Ala Ala Gly Ser Gln Thr Gln Thr Pro Gly Trp Pro
                405                 410                 415
Cys Pro Ser Ser Asp Thr His Leu Pro Thr His Ser Gly Ser Leu
            420                 425                 430
```

<210> 13
<211> 1853
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:/note =
synthetic construct

<400> 13

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aaaacgcagg gagggaggct gtcaccatgc cggcctgctg cagctgcagt gatgttttcc 60
agtatgagac gaacaaagtc actcggatcc agagcatgaa ttatggcacc attaagtggt 120
tcttccacgt gatcatcttt tcctacgttt gctttgctct ggtgagtgac aagctgtacc 180
agcggaaaga gcctgtcatc agttctgtgc acaccaaggt gaaggggata gcagaggtga 240
aagaggagat cgtggagaat ggagtgaaga agttggtgca cagtgtcttt gacaccgcag 300
actacacctt ccctttgcag gggaactctt tcttcgtgat gacaaacttt ctcaaaacag 360
aaggccaaga gcagcggttg tgtcccgagt atcccacccg caggacgctc tgttcctctg 420
accgaggttg taaaaaggga tggatggacc cgcagagcaa aggaattcag accggaaggt 480
gtgtagtgca tgaagggaac cagaagacct gtgaagtctc tgcctggtgc cccatcgagg 540
cagtggaaga ggccccccgg cctgctctct tgaacagtgc cgaaaacttc actgtgctca 600
tcaagaacaa tatcgacttc cccggccaca actacaccac gagaaacatc ctgccaggtt 660
taaacatcac ttgtaccttc cacaagactc agaatccaca gtgtcccatt ttccgactag 720
gagacatctt ccgagaaaca ggcgataatt tttcagatgt ggcaattcag ggcggaataa 780
tgggcattga gatctactgg gactgcaacc tagaccgttg gttccatcac tgccatccca 840
aatacagttt ccgtcgcctt gacgacaaga ccaccaacgt gtccttgtac cctggctaca 900
acttcagata cgccaagtac tacaaggaaa acaatgttga gaaacggact ctgataaaag 960
tcttcgggat ccgttttgac atcctggttt ttggcaccgg aggaaaattt gacattatcc 1020
agctggttgt gtacatcggc tcaaccctct cctacttcgg tctggccgct gtgttcatcg 1080
acttcctcat cgacacttac tccagtaact gctgtcgctc ccatatttat ccctggtgca 1140
agtgctgtca gccctgtgtg gtcaacgaat actactacag gaagaagtgc gagtccattg 1200
tggagccaaa gccgacatta aagtatgtgt cctttgtgga tgaatcccac attaggatgg 1260
tgaaccagca gctactaggg agaagtctgc aagatgtcaa gggccaagaa gtcccaagac 1320
ctgcgatgga cttcacagat ttgtccaggc tgcccctggc cctccatgac acacccccga 1380
ttcctggaca accagaggag atacagctgc ttagaaagga ggcgactcct agatccaggg 1440
atagccccgt ctggtgccag tgtggaagct gcctcccatc tcaactccct gagagccaca 1500
```

```
ggtgcctgga ggagctgtgc tgccggaaaa agccgggggc ctgcatcacc acctcagagc 1560
tgttcaggaa gctggtcctg tccagacacg tcctgcagtt cctcctgctc taccaggagc 1620
ccttgctggc gctggatgtg gattccacca acagccggct gcggcactgt gcctacaggt 1680
gctacgccac ctggcgcttc ggctcccagg acatggctga ctttgccatc ctgcccagct 1740
gctgccgctg gaggatccgg aaagagtttc cgaagagtga agggcagtac agtggcttca 1800
agagtcctta ctgaagccag gcaccgtggc tcacgtctgt aatcccacct ttt         1853

<210> 14
<211> 595
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:/note =
      synthetic construct

<400> 14
Met Pro Ala Cys Cys Ser Cys Ser Asp Val Phe Gln Tyr Glu Thr Asn
 1               5                  10                  15
Lys Val Thr Arg Ile Gln Ser Met Asn Tyr Gly Thr Ile Lys Trp Phe
            20                  25                  30
Phe His Val Ile Ile Phe Ser Tyr Val Cys Phe Ala Leu Val Ser Asp
        35                  40                  45
Lys Leu Tyr Gln Arg Lys Glu Pro Val Ile Ser Ser Val His Thr Lys
    50                  55                  60
Val Lys Gly Ile Ala Glu Val Lys Glu Glu Ile Val Glu Asn Gly Val
65                  70                  75                  80
Lys Lys Leu Val His Ser Val Phe Asp Thr Ala Asp Tyr Thr Phe Pro
                85                  90                  95
Leu Gln Gly Asn Ser Phe Phe Val Met Thr Asn Phe Leu Lys Thr Glu
            100                 105                 110
Gly Gln Glu Gln Arg Leu Cys Pro Glu Tyr Pro Thr Arg Arg Thr Leu
        115                 120                 125
Cys Ser Ser Asp Arg Gly Cys Lys Lys Gly Trp Met Asp Pro Gln Ser
    130                 135                 140
Lys Gly Ile Gln Thr Gly Arg Cys Val Val His Glu Gly Asn Gln Lys
145                 150                 155                 160
Thr Cys Glu Val Ser Ala Trp Cys Pro Ile Glu Ala Val Glu Glu Ala
                165                 170                 175
Pro Arg Pro Ala Leu Leu Asn Ser Ala Glu Asn Phe Thr Val Leu Ile
            180                 185                 190
Lys Asn Asn Ile Asp Phe Pro Gly His Asn Tyr Thr Thr Arg Asn Ile
        195                 200                 205
Leu Pro Gly Leu Asn Ile Thr Cys Thr Phe His Lys Thr Gln Asn Pro
    210                 215                 220
Gln Cys Pro Ile Phe Arg Leu Gly Asp Ile Phe Arg Glu Thr Gly Asp
225                 230                 235                 240
Asn Phe Ser Asp Val Ala Ile Gln Gly Gly Ile Met Gly Ile Glu Ile
                245                 250                 255
Tyr Trp Asp Cys Asn Leu Asp Arg Trp Phe His His Cys His Pro Lys
            260                 265                 270
Tyr Ser Phe Arg Arg Leu Asp Asp Lys Thr Thr Asn Val Ser Leu Tyr
        275                 280                 285
Pro Gly Tyr Asn Phe Arg Tyr Ala Lys Tyr Tyr Lys Glu Asn Asn Val
    290                 295                 300
Glu Lys Arg Thr Leu Ile Lys Val Phe Gly Ile Arg Phe Asp Ile Leu
305                 310                 315                 320
Val Phe Gly Thr Gly Gly Lys Phe Asp Ile Ile Gln Leu Val Val Tyr
                325                 330                 335
Ile Gly Ser Thr Leu Ser Tyr Phe Gly Leu Ala Ala Val Phe Ile Asp
            340                 345                 350
```

```
Phe Leu Ile Asp Thr Tyr Ser Ser Asn Cys Cys Arg Ser His Ile Tyr
        355                 360                 365
Pro Trp Cys Lys Cys Cys Gln Pro Cys Val Val Asn Glu Tyr Tyr Tyr
    370                 375                 380
Arg Lys Lys Cys Glu Ser Ile Val Glu Pro Lys Pro Thr Leu Lys Tyr
385                 390                 395                 400
Val Ser Phe Val Asp Glu Ser His Ile Arg Met Val Asn Gln Gln Leu
                405                 410                 415
Leu Gly Arg Ser Leu Gln Asp Val Lys Gly Gln Glu Val Pro Arg Pro
            420                 425                 430
Ala Met Asp Phe Thr Asp Leu Ser Arg Leu Pro Leu Ala Leu His Asp
        435                 440                 445
Thr Pro Pro Ile Pro Gly Gln Pro Glu Glu Ile Gln Leu Leu Arg Lys
    450                 455                 460
Glu Ala Thr Pro Arg Ser Arg Asp Ser Pro Val Trp Cys Gln Cys Gly
465                 470                 475                 480
Ser Cys Leu Pro Ser Gln Leu Pro Glu Ser His Arg Cys Leu Glu Glu
                485                 490                 495
Leu Cys Cys Arg Lys Lys Pro Gly Ala Cys Ile Thr Thr Ser Glu Leu
            500                 505                 510
Phe Arg Lys Leu Val Leu Ser Arg His Val Leu Gln Phe Leu Leu Leu
        515                 520                 525
Tyr Gln Glu Pro Leu Leu Ala Leu Asp Val Asp Ser Thr Asn Ser Arg
    530                 535                 540
Leu Arg His Cys Ala Tyr Arg Cys Tyr Ala Thr Trp Arg Phe Gly Ser
545                 550                 555                 560
Gln Asp Met Ala Asp Phe Ala Ile Leu Pro Ser Cys Cys Arg Trp Arg
                565                 570                 575
Ile Arg Lys Glu Phe Pro Lys Ser Glu Gly Gln Tyr Ser Gly Phe Lys
            580                 585                 590
Ser Pro Tyr
        595
```

<210> 15
<211> 1312
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:/note =
synthetic construct

<400> 15

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ggatccagtt cgcctgctcc cttccgctcg ctggcttttc cgatgcttgc tgcgcccctg 60
gccgccgctg ccctctcgcc gcctcctacc cctcggagcc gccgcctaag tcgaggagga 120
gagaatgacc gaggtgctgt ggccggctgt ccccaacggg acggacgctg ccttcctggc 180
cggtccgggt tcgtcctggg ggaacagcac ggtcgcctcc actgccgccg tctcctcgtc 240
gttcaaatgc gccttgacca agacgggctt ccagttttac tacctgccgg ctgtctacat 300
cttggtattc atcatcggct tcctgggcaa cagcgtggcc atctggatgt tcgtcttcca 360
catgaagccc tggagcggca tctccgtgta catgttcaat ttggctctgg ccgacttctt 420
gtacgtgctg actctgccag ccctgatctt ctactacttc aataaaacag actggatctt 480
cggggatgcc atgtgtaaac tgcagaggtt catctttcat gtgaacctct atggcagcat 540
cttgtttctg acatgcatca gtgcccaccg gtacagcggt gtggtgtacc ccctcaagtc 600
cctgggccgg ctcaaaaaga agaatgcgat ctgtatcagc gtgctggtgt ggctcattgt 660
ggtggtggcg atctccccca tcctcttcta ctcaggtacc ggggtccgca aaaacaaaac 720
catcacctgt tacgacacca cctcagacga gtacctgcga agttatttca tctacagcat 780
gtgcacgacc gtggccatgt tctgtgtccc cttggtgctg attctgggct gttacggatt 840
aattgtgaga gctttgattt acaaagatct ggacaactct cctctgagga gaaaatcgat 900
ttacctggta atcattgtac tgactgtttt tgctgtgtct tacatccctt tccatgtgat 960
```

gaaaacgatg aacttgaggg cccggcttga ttttcagacc ccagcaatgt gtgctttcaa 1020
tgacagggtt tatgccacgt atcaggtgac aagaggtcta gcaagtctca acagttgtgt 1080
ggaccccatt ctctatttct tggcgggaga tactttcaga aggagactct cccgagccac 1140
aaggaaagct tctagaagaa gtgaggcaaa tttgcaatcc aagagtgaag acatgaccct 1200
caatatttta cctgagttca agcagaatgg agatacaagc ctgtgaaggc acaagaatct 1260
ccaaacacct ctctgttgta atatggtagg atgcttaaca gaatcaagta ct 1312

<210> 16
<211> 373
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:/note =
synthetic construct

<400> 16
Met Thr Glu Val Leu Trp Pro Ala Val Pro Asn Gly Thr Asp Ala Ala
1 5 10 15
Phe Leu Ala Gly Pro Gly Ser Ser Trp Gly Asn Ser Thr Val Ala Ser
20 25 30
Thr Ala Ala Val Ser Ser Ser Phe Lys Cys Ala Leu Thr Lys Thr Gly
35 40 45
Phe Gln Phe Tyr Tyr Leu Pro Ala Val Tyr Ile Leu Val Phe Ile Ile
50 55 60
Gly Phe Leu Gly Asn Ser Val Ala Ile Trp Met Phe Val Phe His Met
65 70 75 80
Lys Pro Trp Ser Gly Ile Ser Val Tyr Met Phe Asn Leu Ala Leu Ala
85 90 95
Asp Phe Leu Tyr Val Leu Thr Leu Pro Ala Leu Ile Phe Tyr Tyr Phe
100 105 110
Asn Lys Thr Asp Trp Ile Phe Gly Asp Ala Met Cys Lys Leu Gln Arg
115 120 125
Phe Ile Phe His Val Asn Leu Tyr Gly Ser Ile Leu Phe Leu Thr Cys
130 135 140
Ile Ser Ala His Arg Tyr Ser Gly Val Val Tyr Pro Leu Lys Ser Leu
145 150 155 160
Gly Arg Leu Lys Lys Lys Asn Ala Ile Cys Ile Ser Val Leu Val Trp
165 170 175
Leu Ile Val Val Val Ala Ile Ser Pro Ile Leu Phe Tyr Ser Gly Thr
180 185 190
Gly Val Arg Lys Asn Lys Thr Ile Thr Cys Tyr Asp Thr Thr Ser Asp
195 200 205
Glu Tyr Leu Arg Ser Tyr Phe Ile Tyr Ser Met Cys Thr Thr Val Ala
210 215 220
Met Phe Cys Val Pro Leu Val Leu Ile Leu Gly Cys Tyr Gly Leu Ile
225 230 235 240
Val Arg Ala Leu Ile Tyr Lys Asp Leu Asp Asn Ser Pro Leu Arg Arg
245 250 255
Lys Ser Ile Tyr Leu Val Ile Ile Val Leu Thr Val Phe Ala Val Ser
260 265 270
Tyr Ile Pro Phe His Val Met Lys Thr Met Asn Leu Arg Ala Arg Leu
275 280 285
Asp Phe Gln Thr Pro Ala Met Cys Ala Phe Asn Asp Arg Val Tyr Ala
290 295 300
Thr Tyr Gln Val Thr Arg Gly Leu Ala Ser Leu Asn Ser Cys Val Asp
305 310 315 320
Pro Ile Leu Tyr Phe Leu Ala Gly Asp Thr Phe Arg Arg Arg Leu Ser
325 330 335
Arg Ala Thr Arg Lys Ala Ser Arg Arg Ser Glu Ala Asn Leu Gln Ser
340 345 350

Lys Ser Glu Asp Met Thr Leu Asn Ile Leu Pro Glu Phe Lys Gln Asn
355 360 365
Gly Asp Thr Ser Leu
370

<210> 17
<211> 2025
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:/note =
synthetic construct

<400> 17
cggcacgagg caccccgaga ggagaagcgc agcgcagtgg cgagaggagc cccttgtggc 60
agcagcacta cctgcccaga aaaatgctgg aggctgggcg tggccccagg cctggggacc 120
tgtttttcct gtttcccgca gagttccctg cagcccggtc caggtccagg cgtgtgcatt 180
catgagtgag gaacccgtgc aggcgctgag catcctgacc tggagagcag gggctggtca 240
gggcgatggc agcagacctg ggcccctgga atgacaccat caatggcacc tgggatgggg 300
atgagctggg ctacaggtgc cgcttcaacg aggacttcaa gtacgtgctg ctgcctgtgt 360
cctacggcgt ggtgtgcgtg cttgggctgt gtctgaacgc cgtggcgctc tacatcttct 420
tgtgccgcct caagacctgg aatgcgtcca ccacatatat gttccacctg gctgtgtctg 480
atgcactgta tgcggcctcc ctgccgctgc tggtctatta ctacgcccgc ggcgaccact 540
ggcccttcag cacggtgctc tgcaagctgg tgcgcttcct cttctacacc aacctttact 600
gcagcatcct cttcctcacc tgcatcagcg tgcaccggtg tctgggcgtc ttacgacctc 660
tgcgctccct gcgctggggc cgggcccgct acgctcgccg ggtggccggg gccgtgtggg 720
tgttggtgct ggcctgccag gcccccgtgc tctactttgt caccaccagc gcgcgcgggg 780
gccgcgtaac ctgccacgac acctcggcac ccgagctctt cagccgcttc gtggcctaca 840
gctcagtcat gctgggcctg ctcttcgcgg tgccctttgc cgtcatcctt gtctgttacg 900
tgctcatggc tcggcgactg ctaaagccag cctacgggac ctcgggcggc ctccctaggg 960
ccaagcgcaa gtccgtgcgc accatcgccg tggtgctggc tgtcttcgcc ctctgcttcc 1020
tgccattcca cgtcacccgc accctctact actccttccg ctcgctggac ctcagctgcc 1080
acaccctcaa cgccatcaac atggcctaca aggttacccg gccgctggcc agtgctaaca 1140
gttgccttga ccccgtgctc tacttcctgg ctgggcagag gctcgtacgc tttgcccgag 1200
atgccaagcc acccactggc cccagccctg ccaccccggc tcgccgcagg ctgggcctgc 1260
gcagatccga cagaactgac atgcagagga taggagatgt gttgggcagc agtgaggact 1320
tcaggcggac agagtccacg ccggctggta gcgagaacac taaggacatt cggctgtagg 1380
agcagaacac ttcagcctgt gcaggtttat attgggaagc tgtagaggac caggacttgt 1440
gcagacgcca cagtctcccc agatatggac catcagtgac tcatgctgga tgaccccatg 1500
ctccgtcatt tgacaggggc tcaggatatt cactctgtgg tccagagtca actgttccca 1560
taacccctag tcatcgtttg tgtgtataag ttgggggaat taagtttcaa gaaaggcaag 1620
agctcaaggt caatgacacc cctggcctga ctcccatgca agtagctggc tgtactgcca 1680
aggtacctag gttggagtcc agcctaatca agtcaaatgg agaaacaggc ccagagagga 1740
aggtggctta ccaagatcac ataccagagt ctggagctga gctacctggg gtgggggcca 1800
agtcacaggt tggccagaaa accctggtaa gtaatgaggg ctgagtttgc acagtggtct 1860
ggaatggact gggtgccacg gtggacttag ctctgaggag taccccagc ccaagagatg 1920
aacatctggg gactaatatc atagacccat ctggaggctc ccatgggcta ggagcagtgt 1980
gaggctgtaa cttatactaa aggttgtgtt gcctgctaaa aaaaa 2025

<210> 18
<211> 377
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:/note =
synthetic construct

<400> 18

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Met Ala Ala Asp Leu Gly Pro Trp Asn Asp Thr Ile Asn Gly Thr Trp
 1               5                  10                  15
Asp Gly Asp Glu Leu Gly Tyr Arg Cys Arg Phe Asn Glu Asp Phe Lys
            20                  25                  30
Tyr Val Leu Leu Pro Val Ser Tyr Gly Val Val Cys Val Leu Gly Leu
        35                  40                  45
Cys Leu Asn Ala Val Ala Leu Tyr Ile Phe Leu Cys Arg Leu Lys Thr
    50                  55                  60
Trp Asn Ala Ser Thr Thr Tyr Met Phe His Leu Ala Val Ser Asp Ala
65                  70                  75                  80
Leu Tyr Ala Ala Ser Leu Pro Leu Leu Val Tyr Tyr Tyr Ala Arg Gly
                85                  90                  95
Asp His Trp Pro Phe Ser Thr Val Leu Cys Lys Leu Val Arg Phe Leu
            100                 105                 110
Phe Tyr Thr Asn Leu Tyr Cys Ser Ile Leu Phe Leu Thr Cys Ile Ser
        115                 120                 125
Val His Arg Cys Leu Gly Val Leu Arg Pro Leu Arg Ser Leu Arg Trp
    130                 135                 140
Gly Arg Ala Arg Tyr Ala Arg Arg Val Ala Gly Ala Val Trp Val Leu
145                 150                 155                 160
Val Leu Ala Cys Gln Ala Pro Val Leu Tyr Phe Val Thr Thr Ser Ala
                165                 170                 175
Arg Gly Gly Arg Val Thr Cys His Asp Thr Ser Ala Pro Glu Leu Phe
            180                 185                 190
Ser Arg Phe Val Ala Tyr Ser Ser Val Met Leu Gly Leu Leu Phe Ala
        195                 200                 205
Val Pro Phe Ala Val Ile Leu Val Cys Tyr Val Leu Met Ala Arg Arg
    210                 215                 220
Leu Leu Lys Pro Ala Tyr Gly Thr Ser Gly Gly Leu Pro Arg Ala Lys
225                 230                 235                 240
Arg Lys Ser Val Arg Thr Ile Ala Val Val Leu Ala Val Phe Ala Leu
                245                 250                 255
Cys Phe Leu Pro Phe His Val Thr Arg Thr Leu Tyr Tyr Ser Phe Arg
            260                 265                 270
Ser Leu Asp Leu Ser Cys His Thr Leu Asn Ala Ile Asn Met Ala Tyr
        275                 280                 285
Lys Val Thr Arg Pro Leu Ala Ser Ala Asn Ser Cys Leu Asp Pro Val
    290                 295                 300
Leu Tyr Phe Leu Ala Gly Gln Arg Leu Val Arg Phe Ala Arg Asp Ala
305                 310                 315                 320
Lys Pro Pro Thr Gly Pro Ser Pro Ala Thr Pro Ala Arg Arg Arg Leu
                325                 330                 335
Gly Leu Arg Arg Ser Asp Arg Thr Asp Met Gln Arg Ile Gly Asp Val
            340                 345                 350
Leu Gly Ser Ser Glu Asp Phe Arg Arg Thr Glu Ser Thr Pro Ala Gly
        355                 360                 365
Ser Glu Asn Thr Lys Asp Ile Arg Leu
    370                 375
```

<210> 19
<211> 1163
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:/note =
synthetic construct

<400> 19
ggcgcttcac ccagtaaaga gggaccatga gcatggccaa cttcacgggg gggaggaact 60

cgtgcacctt ccatgaggaa ttcaagcagg tcctgctgcc cctggtctac tcagtggtgt 120
tcctactggg gctgccactc aatgccgttg tcattgggca gatctggctg gcccgcaagg 180
cgttgacccg caccaccatc tacatgctga acctggccat ggccgacctg ctttatgtct 240
gctccctccc tctcctcatc tacaactaca cccagaagga ttactggccc tttggggact 300
tcacctgcaa attcgtccgc ttccagttct acaccaacct gcacggcagc atcctcttcc 360
tcacctgcat cagcgtccag cgctacatgg ggatctgcca ccccttggcc tcgtggcaca 420
aaaagaaggg aaagaagctg acgtggctgg tgtgtgctgc cgtgtggttc atcgtcatcg 480
cccagtgcct gcccaccttt gtcttcgcct ccaccggcac gcagaggaat cgcactgtct 540
gctatgacct gagccccccg gaccgctcca catcctactt cccctatggc atcacgttga 600
ccatcactgg cttcctgctg cccttcgcag ccatcctggc ctgctactgc agcatggccc 660
gcatcctgtg ccagaaagac gagctgattg gcttggcggt gcacaagaag aaggacaagg 720
ccgtgcgcat gatcatcatc gttgtcatcg tcttctccat cagcttcttc cccttccacc 780
tcaccaagac catctacctg atcgtccgct cctcagccag cttgccctgc cctaccctgc 840
aggcttttgc cattgcctac aagtgcacgc ggccctttgc cagcatgaac agcgtcctcg 900
accccatcct cttctacttc acccagcgca agtttcgtga gagcacccgc tatctcctgg 960
acaagatgag ctccaagtgg cggcaagacc actgcatcag ctacggctcc taggtggacg 1020
aggccacctc ggtgtcaccg gggctgggca tggagcaatt tgggttgaag ctgcatggtg 1080
cggagatggg gatgagccca gagtgctgcg ggtgccccat ctctggaggt gttggagatt 1140
agattggatg gggctctggg ccc 1163

<210> 20
<211> 328
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:/note =
synthetic construct

<400> 20
Met Ser Met Ala Asn Phe Thr Gly Gly Arg Asn Ser Cys Thr Phe His
1 5 10 15
Glu Glu Phe Lys Gln Val Leu Leu Pro Leu Val Tyr Ser Val Val Phe
20 25 30
Leu Leu Gly Leu Pro Leu Asn Ala Val Val Ile Gly Gln Ile Trp Leu
35 40 45
Ala Arg Lys Ala Leu Thr Arg Thr Thr Ile Tyr Met Leu Asn Leu Ala
50 55 60
Met Ala Asp Leu Leu Tyr Val Cys Ser Leu Pro Leu Leu Ile Tyr Asn
65 70 75 80
Tyr Thr Gln Lys Asp Tyr Trp Pro Phe Gly Asp Phe Thr Cys Lys Phe
85 90 95
Val Arg Phe Gln Phe Tyr Thr Asn Leu His Gly Ser Ile Leu Phe Leu
100 105 110
Thr Cys Ile Ser Val Gln Arg Tyr Met Gly Ile Cys His Pro Leu Ala
115 120 125
Ser Trp His Lys Lys Lys Gly Lys Lys Leu Thr Trp Leu Val Cys Ala
130 135 140
Ala Val Trp Phe Ile Val Ile Ala Gln Cys Leu Pro Thr Phe Val Phe
145 150 155 160
Ala Ser Thr Gly Thr Gln Arg Asn Arg Thr Val Cys Tyr Asp Leu Ser
165 170 175
Pro Pro Asp Arg Ser Thr Ser Tyr Phe Pro Tyr Gly Ile Thr Leu Thr
180 185 190
Ile Thr Gly Phe Leu Leu Pro Phe Ala Ala Ile Leu Ala Cys Tyr Cys
195 200 205
Ser Met Ala Arg Ile Leu Cys Gln Lys Asp Glu Leu Ile Gly Leu Ala
210 215 220
Val His Lys Lys Lys Asp Lys Ala Val Arg Met Ile Ile Ile Val Val
225 230 235 240

```
Ile Val Phe Ser Ile Ser Phe Phe Pro Phe His Leu Thr Lys Thr Ile
                245                 250                 255
Tyr Leu Ile Val Arg Ser Ser Ala Ser Leu Pro Cys Pro Thr Leu Gln
            260                 265                 270
Ala Phe Ala Ile Ala Tyr Lys Cys Thr Arg Pro Phe Ala Ser Met Asn
        275                 280                 285
Ser Val Leu Asp Pro Ile Leu Phe Tyr Phe Thr Gln Arg Lys Phe Arg
    290                 295                 300
Glu Ser Thr Arg Tyr Leu Leu Asp Lys Met Ser Ser Lys Trp Arg Gln
305                 310                 315                 320
Asp His Cys Ile Ser Tyr Gly Ser
                325
```

<210> 21
<211> 1429
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:/note =
synthetic construct

<400> 21

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ctccctagtg cttcaaccac tgctctccct gctctacttt ttttgctcca gctcagggat 120
gggggtgggc agggaaatcc tgccaccctc acttctcccc ttcccatctc caggggggcc 180
atggccagta cagagtcctc cctgttgaga tccctaggcc tcagcccagg tcctggcagc 240
agtgaggtgg agctggactg ttggtttgat gaggatttca agttcatcct gctgcctgtg 300
agctatgcag ttgtctttgt gctgggcttg ggccttaacg ccccaaccct atggctcttc 360
atcttccgcc tccgaccctg ggatgcaacg gccacctaca tgttccacct ggcattgtca 420
gacaccttgt atgtgctgtc gctgcccacc ctcatctact attatgcagc ccacaaccac 480
tggccctttg gcactgagat ctgcaagttc gtccgctttc ttttctattg gaacctctac 540
tgcagtgtcc ttttcctcac ctgcatcagc gtgcaccgct acctgggcat ctgccaccca 600
cttcgggcac tacgctgggg ccgccctcgc ctcgcaggcc ttctctgcct ggcagtttgg 660
ttggtcgtag ccggctgcct cgtgcccaac ctgttctttg tcacaaccag caacaaaggg 720
accaccgtcc tgtgccatga caccactcgg cctgaagagt ttgaccacta tgtgcacttc 780
agctcggcgg tcatggggct gctctttggc gtgccctgcc tggtcactct tgtttgctat 840
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ctccgctctc tccgcaccat agctgtggtg ctgactgtct ttgctgtctg cttcgtgcct 960
ttccacatca cccgcaccat ttactacctg gccaggctgt tggaagctga ctgccgagta 1020
ctgaacattg tcaacgtggt ctataaagtg actcggcccc tggccagtgc caacagctgc 1080
ctggatcctg tgctctactt gctcactggg gacaaatatc gacgtcagct ccgtcagctc 1140
tgtggtggtg gcaagcccca gccccgcacg gctgcctctt ccctggcact agtgtccctg 1200
cctgaggata gcagctgcag gtgggcggcc acccccccagg acagtagctg ctctactcct 1260
agggcagata gattgtaaca cgggaagccg ggaagtgaga gaaaagggga tgagtgcagg 1320
gcagaggtga gggaacccaa tagtgatacc tggtaaggtg cttcttcctc ttttccaggc 1380
tctggagaga agccctcacc ctgagggttg ccagggaggc agggatatc              1429
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<210> 22
<211> 365
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:/note =
synthetic construct

<400> 22

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            20                  25                  30
Phe Lys Phe Ile Leu Leu Pro Val Ser Tyr Ala Val Val Phe Val Leu
        35                  40                  45
Gly Leu Gly Leu Asn Ala Pro Thr Leu Trp Leu Phe Ile Phe Arg Leu
    50                  55                  60
Arg Pro Trp Asp Ala Thr Ala Thr Tyr Met Phe His Leu Ala Leu Ser
65                  70                  75                  80
Asp Thr Leu Tyr Val Leu Ser Leu Pro Thr Leu Ile Tyr Tyr Tyr Ala
                85                  90                  95
Ala His Asn His Trp Pro Phe Gly Thr Glu Ile Cys Lys Phe Val Arg
            100                 105                 110
Phe Leu Phe Tyr Trp Asn Leu Tyr Cys Ser Val Leu Phe Leu Thr Cys
        115                 120                 125
Ile Ser Val His Arg Tyr Leu Gly Ile Cys His Pro Leu Arg Ala Leu
    130                 135                 140
Arg Trp Gly Arg Pro Arg Leu Ala Gly Leu Leu Cys Leu Ala Val Trp
145                 150                 155                 160
Leu Val Val Ala Gly Cys Leu Val Pro Asn Leu Phe Phe Val Thr Thr
                165                 170                 175
Ser Asn Lys Gly Thr Thr Val Leu Cys His Asp Thr Thr Arg Pro Glu
            180                 185                 190
Glu Phe Asp His Tyr Val His Phe Ser Ser Ala Val Met Gly Leu Leu
        195                 200                 205
Phe Gly Val Pro Cys Leu Val Thr Leu Val Cys Tyr Gly Leu Met Ala
    210                 215                 220
Arg Arg Leu Tyr Gln Pro Leu Pro Gly Ser Ala Gln Ser Ser Ser Arg
225                 230                 235                 240
Leu Arg Ser Leu Arg Thr Ile Ala Val Val Leu Thr Val Phe Ala Val
                245                 250                 255
Cys Phe Val Pro Phe His Ile Thr Arg Thr Ile Tyr Tyr Leu Ala Arg
            260                 265                 270
Leu Leu Glu Ala Asp Cys Arg Val Leu Asn Ile Val Asn Val Val Tyr
        275                 280                 285
Lys Val Thr Arg Pro Leu Ala Ser Ala Asn Ser Cys Leu Asp Pro Val
    290                 295                 300
Leu Tyr Leu Leu Thr Gly Asp Lys Tyr Arg Arg Gln Leu Arg Gln Leu
305                 310                 315                 320
Cys Gly Gly Gly Lys Pro Gln Pro Arg Thr Ala Ala Ser Ser Leu Ala
                325                 330                 335
Leu Val Ser Leu Pro Glu Asp Ser Ser Cys Arg Trp Ala Ala Thr Pro
            340                 345                 350
Gln Asp Ser Ser Cys Ser Thr Pro Arg Ala Asp Arg Leu
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<211> 1571
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<213> Artificial Sequence

<220>
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tcacggactg caagcgaggc acttgctaac tcttggataa caagacctct gccagaagaa 180
ccatggcttt ggaaggcgga gttcaggctg aggagatggg tgcggtcctc agtgagcccc 240
tgcctccctg aacataggaa acccacctgg gcagccatgg aatgggacaa tggcacaggc 300

caggctctgg gcttgccacc caccacctgt gtctaccgcg agaacttcaa gcaactgctg 360
ctgccacctg tgtattcggc ggtgctggcg gctggcctgc cgctgaacat ctgtgtcatt 420
acccagatct gcacgtcccg ccgggccctg acccgcacgg ccgtgtacac cctaaacctt 480
gctctggctg acctgctata tgcctgctcc ctgcccctgc tcatctacaa ctatgcccaa 540
ggtgatcact ggcccttttgg cgacttcgcc tgccgcctgg tccgcttcct cttctatgcc 600
aacctgcacg gcagcatcct cttcctcacc tgcatcagct tccagcgcta cctgggcatc 660
tgccacccgc tggcccccctg gcacaaacgt gggggccgcc gggctgcctg gctagtgtgt 720
gtagccgtgt ggctggccgt gacaacccag tgcctgccca cagccatctt cgctgccaca 780
ggcatccagc gtaaccgcac tgtctgctat gacctcagcc cgcctgccct ggccacccac 840
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gccatcagct tcctgccttt tcacatcacc aagacagcct acctggcagt gcgctcgacg 1080
ccgggcgtcc cctgcactgt attggaggcc tttgcagcgg cctacaaagg cacgcggccg 1140
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cgccggcgac cacatgagct cctacagaaa ctcacagcca aatggcagag gcagggtcgc 1260
tgagtcctcc aggtcctggg cagccttcat atttgccatt gtgtccgggg caccaggagc 1320
cccaccaacc ccaaaccatg cggagaatta gagttcagct cagctgggca tggagttaag 1380
atccctcaca ggacccagaa gctcaccaaa aactatttct tcagcccctt ctctggccca 1440
gaccctgtgg gcatggagat ggacagacct gggcctggct cttgagaggt cccagtcagc 1500
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tcaaaaaaaa a 1571

<210> 24
<211> 328
<212> PRT
<213> Artificial Sequence

<220>
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synthetic construct

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35 40 45
Thr Gln Ile Cys Thr Ser Arg Arg Ala Leu Thr Arg Thr Ala Val Tyr
50 55 60
Thr Leu Asn Leu Ala Leu Ala Asp Leu Leu Tyr Ala Cys Ser Leu Pro
65 70 75 80
Leu Leu Ile Tyr Asn Tyr Ala Gln Gly Asp His Trp Pro Phe Gly Asp
85 90 95
Phe Ala Cys Arg Leu Val Arg Phe Leu Phe Tyr Ala Asn Leu His Gly
100 105 110
Ser Ile Leu Phe Leu Thr Cys Ile Ser Phe Gln Arg Tyr Leu Gly Ile
115 120 125
Cys His Pro Leu Ala Pro Trp His Lys Arg Gly Gly Arg Arg Ala Ala
130 135 140
Trp Leu Val Cys Val Ala Val Trp Leu Ala Val Thr Thr Gln Cys Leu
145 150 155 160
Pro Thr Ala Ile Phe Ala Ala Thr Gly Ile Gln Arg Asn Arg Thr Val
165 170 175
Cys Tyr Asp Leu Ser Pro Pro Ala Leu Ala Thr His Tyr Met Pro Tyr
180 185 190
Gly Met Ala Leu Thr Val Ile Gly Phe Leu Leu Pro Phe Ala Ala Leu
195 200 205
Leu Ala Cys Tyr Cys Leu Leu Ala Cys Arg Leu Cys Arg Gln Asp Gly
210 215 220

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Pro Ala Glu Pro Val Ala Gln Glu Arg Arg Gly Lys Ala Ala Arg Met
225                 230                 235                 240
Ala Val Val Val Ala Ala Ala Phe Ala Ile Ser Phe Leu Pro Phe His
                245                 250                 255
Ile Thr Lys Thr Ala Tyr Leu Ala Val Arg Ser Thr Pro Gly Val Pro
            260                 265                 270
Cys Thr Val Leu Glu Ala Phe Ala Ala Ala Tyr Lys Gly Thr Arg Pro
        275                 280                 285
Phe Ala Ser Ala Asn Ser Val Leu Asp Pro Ile Leu Phe Tyr Phe Thr
    290                 295                 300
Gln Lys Lys Phe Arg Arg Arg Pro His Glu Leu Leu Gln Lys Leu Thr
305                 310                 315                 320
Ala Lys Trp Gln Arg Gln Gly Arg
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<210> 25
<211> 1116
<212> DNA
<213> Artificial Sequence

<220>
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synthetic construct

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gccagcaatg gcctggccct gtaccgcttc agcatccgga agcagcgccc atggcacccc 180
gccgtggtct tctctgtcca gctggcagtc agcgacctgc tctgcgctct gacgctgccc 240
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ctggagcgct tcctcttcac ctgcaacctg ctgggcagcg tcatcttcat cacctgcatc 360
agcctcaacc gctacctggg catcgtgcac cccttcttcg cccgaagcca cctgcgaccc 420
aagcacgcct gggccgtgag cgctgccggc tgggtcctgg ccgccctgct ggccatgccc 480
acactcagct tctcccacct gaagaggccg cagcaggggg cgggcaactg cagcgtggcc 540
aggcccgagg cctgcatcaa gtgtctgggg acagcagacc acgggctggc ggcctacaga 600
gcgtatagcc tggtgctggc ggggttgggc tgcggcctgc cgctgctgct cacgctggca 660
gcctacggcg ccctcgggcg ggccgtgcta cgcagcccag gcatgactgt ggccgagaag 720
ctgcgtgtgg cagcgttggt ggccagtggt gtggccctct acgccagctc ctatgtgccc 780
taccacatca tgcgggtgct caacgtggat gctcggcggc gctggagcac ccgctgcccg 840
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caggtgatgc ggggcctcat gcccctggcc ttctgtgtcc accctctact ctacatggcc 960
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<210> 26
<211> 371
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence:/note =
synthetic construct

<400> 26
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 1               5                  10                  15
Asp Asp Lys Leu Ser Gly Phe Gln Gly Asp Phe Leu Trp Pro Ile Leu
            20                  25                  30
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Val Val Glu Phe Leu Val Ala Val Ala Ser Asn Gly Leu Ala Leu Tyr
        35                  40                  45
Arg Phe Ser Ile Arg Lys Gln Arg Pro Trp His Pro Ala Val Val Phe
    50                  55                  60
Ser Val Gln Leu Ala Val Ser Asp Leu Leu Cys Ala Leu Thr Leu Pro
65                  70                  75                  80
Pro Leu Ala Ala Tyr Leu Tyr Pro Pro Lys His Trp Arg Tyr Gly Glu
                85                  90                  95
Ala Ala Cys Arg Leu Glu Arg Phe Leu Phe Thr Cys Asn Leu Leu Gly
            100                 105                 110
Ser Val Ile Phe Ile Thr Cys Ile Ser Leu Asn Arg Tyr Leu Gly Ile
        115                 120                 125
Val His Pro Phe Phe Ala Arg Ser His Leu Arg Pro Lys His Ala Trp
    130                 135                 140
Ala Val Ser Ala Ala Gly Trp Val Leu Ala Ala Leu Leu Ala Met Pro
145                 150                 155                 160
Thr Leu Ser Phe Ser His Leu Lys Arg Pro Gln Gln Gly Ala Gly Asn
                165                 170                 175
Cys Ser Val Ala Arg Pro Glu Ala Cys Ile Lys Cys Leu Gly Thr Ala
            180                 185                 190
Asp His Gly Leu Ala Ala Tyr Arg Ala Tyr Ser Leu Val Leu Ala Gly
        195                 200                 205
Leu Gly Cys Gly Leu Pro Leu Leu Leu Thr Leu Ala Ala Tyr Gly Ala
    210                 215                 220
Leu Gly Arg Ala Val Leu Arg Ser Pro Gly Met Thr Val Ala Glu Lys
225                 230                 235                 240
Leu Arg Val Ala Ala Leu Val Ala Ser Gly Val Ala Leu Tyr Ala Ser
                245                 250                 255
Ser Tyr Val Pro Tyr His Ile Met Arg Val Leu Asn Val Asp Ala Arg
            260                 265                 270
Arg Arg Trp Ser Thr Arg Cys Pro Ser Phe Ala Asp Ile Ala Gln Ala
        275                 280                 285
Thr Ala Ala Leu Glu Leu Gly Pro Tyr Val Gly Tyr Gln Val Met Arg
    290                 295                 300
Gly Leu Met Pro Leu Ala Phe Cys Val His Pro Leu Leu Tyr Met Ala
305                 310                 315                 320
Ala Val Pro Ser Leu Gly Cys Cys Cys Arg His Cys Pro Gly Tyr Arg
                325                 330                 335
Asp Ser Trp Asn Pro Glu Asp Ala Lys Ser Thr Gly Gln Ala Leu Pro
            340                 345                 350
Leu Asn Ala Thr Ala Ala Pro Lys Pro Ser Glu Pro Gln Ser Arg Glu
        355                 360                 365
Leu Ser Gln
    370
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(72) Inventors; and

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(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.

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Published:
— *with international search report*
— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments*

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For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: PURINERGIC MODULATION OF SMELL

(57) Abstract: Disclosed are compositions and methods for modulating odor sensitivity, as well as screening methods for detecting compounds that modulate odor sensitivity.

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US03/37389

A. CLASSIFICATION OF SUBJECT MATTER
IPC(7) : A01N 43/04; A61K 31/70
US CL : 514/42, 43, 44, 45, 46, 47
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
U.S. : 514/42, 43, 44, 45, 46, 47

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
WEST, CAPLUS, USPATFULL

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 6,063,596 A (LAL et al.) 16 May 2000 (16.05.2000).	1-66
A	US 2001/0025099 A1 (ELSHOURBAGY et al.) 27 September 2001 (27.09.2001).	25-50

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

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Date of the actual completion of the international search
20 August 2004 (20.08.2004)

Date of mailing of the international search report
02 SEP 2004

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